THE DEVELOPMENT OF THE CONDUCTING SYSTEM

IN THE HEART OF THE SHEEP

being

A Thesis for the degree of M.D.

of the University of Edinburgh

by

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C O N T E N T S

I N T R O D U C T I O N

In view of the great functional importance of the cardiac conducting tissue, it has been the subject of a great deal of research throughout this century. But the development of these structures has not received as much attention as the other aspects of its anatomy and physiology.

A perusal of the literature on this subject reveals a difference of opinion regarding the origin of the conducting tissue. On chiefly phylogenetic grounds, Keith & Flack (1907), Keith & Mackenzie (1910) and Mackenzie (1913) regarded the mammalian conducting tissue as a remnant of the more extensive junctional tissues of the cold-blooded vertebrates. This view was generally held and received ontogenetic support from Mall (1912) in his general account of the development of the human heart. Mall describes the development of the atrio-ventricular valves by a process of undermining of the muscular wall leaving the common trunk of the atrio-ventricular bundle as the only remaining connection; but/

but Mall did state that the terminal branches of the bundle were differentiated 'in situ' from the inner, spongy layer of the myocardium. Similar conclusions were reached by Waterston (1918) in another general description of the development of the human heart.

The first embryological investigation directed specially at the conducting tissue was that of Retzer (1908) who, working on pig embryos, reached the conclusion that the conducting tissue was developed from the sinus musculature and he introduced the term sino-ventricular bundle. This opinion of Retzer was tentatively supported by Tandler (1912) on the basis of observations on two human embryos.

The first worker to regard the sinu-atrial node as a new development and not a reduced portion of the sinu-atrial musculature, appears to have been Steinon who published his account of its embryology in 1926. In an earlier paper, Steinon (1925) had investigated the origin of the Purkinje system in man and rabbit; he concluded that this tissue was developed from the spongy layer of the myocardium, and he attributed the origin of the atrio-ventricular bundle to an extension of this tissue into the atria.

An account of the development of the whole system was published by Shaner (1929), who reached the conclusion that the atrio-ventricular bundle develops as a new/

new growth from the atrio-ventricular node. Shaner carried out his investigation on the calf, but his opinion on the origin of the bundle was endorsed by Walls (1947) who studied the human development.

Another mode of development was suggested by Sanabria (1935) who made a very full investigation on human, sheep and rabbit embryos. Sanabria attributed the common trunk of the bundle to the differentiated posterior wall of the atrial canal while he considered that the ventricular Purkinje network was formed "par un phénomène d'induction" which emanated from the cells of the common trunk.

There has been an account of the development in the calf, Calcagno (1941a,b), in which the node and the bundle are described as appearing separately and fusing secondarily.

These conflicting opinions, regarding the origin of the conducting tissue have acquired a greater interest since Davies has advanced his theory that the conducting tissue is a neomorphic development of the warm-blooded vertebrates, Davies & Francis (1941, 1946) and Davies (1942). The present research was carried out with this problem in mind, and the work was performed on the sheep because, being an ungulate, it has a well differentiated conducting tissue.

Since this work has been completed and during the/

the preparation of this thesis, Field (1951 a) has published his observations on the development of the conducting tissue in the sheep. Field's results were not available while this work was being carried out, so that the investigations can be regarded as entirely independent.

MATERIAL AND METHODS

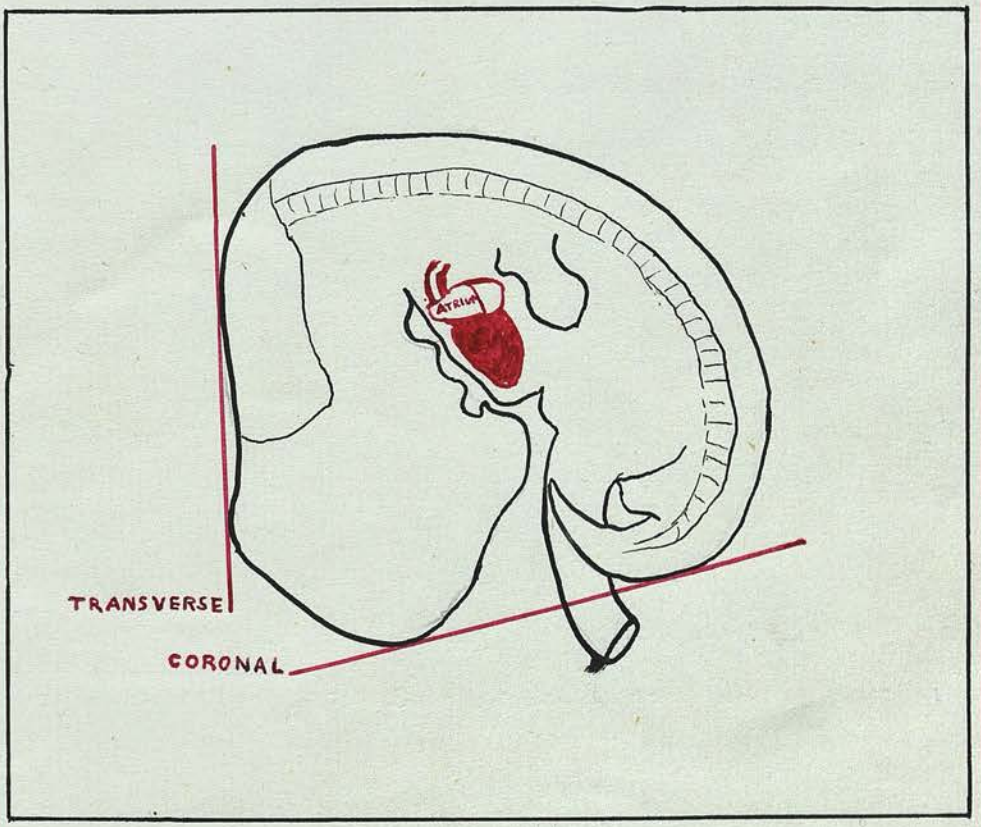
This investigation was carried out, in the Department of Anatomy, University of Edinburgh, on pre-natal and adult sheep material obtained from the Edinburgh abbatoir.

Fixation was effected immediately after death, in every case while the tissue was still warm and in most cases while the heart was still beating. The very young embryos were fixed, whole, in Bouin's fluid; the older embryos were fixed by injection of the umbilical artery with either Bouin's fluid or 10% Formalin. The adult hearts were fixed in Susa, Bouin, Formol-Acetic-Alcohol or in 10% Formalin. Most of the embryonic tissue had been stored in the fixative for 12 months before the present investigation was started.

When the length of the embryo made the task of mounting the whole embryo too tedious, the thorax alone was embedded. In the later stages it was necessary to dissect out the heart prior to embedding.

Particularly in the younger stages it was found helpful to have series cut in different planes for any given stage of development. In the embryos of less than 20 mm. CR length, the coronal plane of section traverses the heart from apex to base. A number of series were cut in this plane/

plane so that the structure of the heart could be compared with the older specimens, of which the majority were sectioned transverse to the heart commencing at the apex. Text-Figure(1) has been included so that there may be no dubiety as to the planes of sectioning in the younger embryos. Although Walls (1947) did not find the sagittal plane of section very helpful in the study of the cardiac conducting tissue, the embryos of the present series which were cut in this plane did show certain points with great clarity.



Text-Figure (1).

The/

The staining method, which has been used chiefly, was Hollande's Chlorcarmin Method as quoted by Lee (1928). This stain was found to give a clear picture of the nuclear structure and of the myofibrils; a further advantage of this stain was that the resulting black and white section was very suitable for photomicrography. Of the other staining methods which were tried, Heidenhain's Iron Haematoxylin without a counterstain was by far the most suitable, and, in fact, gives a picture very similar to that obtained by Hollande's method. The collagen of the fibrous atrio-ventricular ring of the heart was demonstrated by Heidenhain's Azocarmine-Aniline Blue method. The most specific stain for young elastic tissue was found to be Weigert's Resorcin-Fuchsin method.

Although the glycogen content of the adult Purkinje fibres after fixation in Susa or Formol-Acetic - Alcohol, could be readily demonstrated by Bauer's method, as described by Herman (1949); the glycogen in the Purkinje fibres of the foetal hearts could not be demonstrated by the same technique. This was felt to be due to improper fixation, 10% Formol, and prolonged storage, 12 months. Consequently this present work will not include any comments on the appearance of glycogen in the conducting tissue.

As/

As listed below, 40 pre-natal and 6 adult specimens have been examined. All the measurements were made while the specimens were in their fixatives.

	Crown Rump Length	Fixed	Plane of Section	Stained	Comments
1.	5.4 mm.	Bouin's Fluid	Transverse Section to embryo	Hollande	Complete
2.	6.5 mm.	Bouin's Fluid	Transverse Section to embryo	Weigert's Iron Haematoxylin and alcoholic eosin	Complete
3.	6.7 mm.	Bouin's Fluid	Transverse Section to embryo	Hollande	Complete
4.	8.4 mm.	Bouin's Fluid	Sagittal	Hollande	Complete
5.	9.1 mm.	Bouin's Fluid	Coronal to embryo	Hollande	Complete
6.	9.7 mm.	Bouin's Fluid	Transverse Section to embryo	Masson's trichome	Complete
7.	10.1 mm.	Bouin's Fluid	Transverse Section to embryo	Weigert's Iron Haematoxylin and alcoholic eosin	Complete
8.	10.6 mm.	Bouin's Fluid	Transverse Section to embryo	Heidenhain's Iron Haematoxylin	Complete
9.	11.5 mm.	Bouin's Fluid	Transverse Section to embryo	Heidenhain's Iron Haematoxylin	Complete
10.	11.6 mm.	Bouin's Fluid	Transverse Section to embryo	Hollande	Heart section only
11.	11.6 mm.	Bouin's Fluid	Transverse Section to embryo	Weigert's Iron Haematoxylin and alcoholic eosin	Complete
12.	11.7 mm.	Bouin's Fluid	Coronal to embryo	Hollande	Complete
13.	12.6 mm.	10% Formol	Sagittal	Hollande	Complete
14.	14.7 mm.	Bouin's Fluid	Transverse Section to embryo	Weigert's Iron Haematoxylin and alcoholic eosin	Complete
15.	16.6 mm.	Bouin's Fluid	Coronal to embryo	Hollande	Complete
16.	18.4 mm.	Bouin's Fluid	Sagittal	Hollande	Complete
17.	20.1 mm.	Bouin's Fluid	Transverse Section to heart	Hollande	Thorax only
18.	22.8 mm.	Bouin's Fluid	Transverse Section to heart	Hollande	Thorax only
19.	23.6 mm.	10% Formol	Transverse Section to heart	Weigert's Iron Haematoxylin and alcoholic eosin	Thorax only
20.	25.0 mm.	10% Formol	Sagittal	Hollande	Mediastinum only
21.	28.0 mm.	10% Formol	Transverse Section to heart	Hollande	Heart only
22.	29.7 mm.	10% Formol	Transverse Section to heart	Weigert's Iron haematoxylin and alcoholic eosin	Thorax only
23.	32.2 mm.	Bouin's Fluid	Transverse Section to heart	Hollande Heidenhain's Iron Haematoxylin Heidenhain's Azocarmine-Aniline blue	Thorax only

	Crown Rump Length	Fixed	Plane of Section	Stained	Comments
24.	38.6 mm.	10% Formol	Transverse Section to heart	Hollande Heidenhain's Iron Haematoxylin Heidenhain's Azocarmine-Aniline blue	Thorax only
25.	41.9 mm.	10% Formol	Transverse Section to heart	Hollande	Thorax only
26.	43.3 mm.	Bouin's Fluid	Transverse Section to heart	Weigert's Iron Haematoxylin and alcoholic eosin	Thorax only
27.	44.0 mm.	10% Formol	Sagittal	Hollande Weigert's Iron Haematoxylin	Mediastinum only
28.	63. mm.	10% Formol	Coronal to heart	Weigert's Iron Haematoxylin and alcoholic eosin	Heart only
29.	71. mm.	10% Formol	Transverse Section to heart	Hollande	Heart complete
30.	92. mm.	10% Formol	Transverse Section to heart	Hollande	Heart complete
31.	126. mm.	10% Formol	Transverse Section to heart	Hollande	Interrupted sections
32.	138. mm.	10% Formol	Transverse Section to heart	Hollande Heidenhain's Iron Haematoxylin	Heart complete
33.	155. mm.	10% Formol	Transverse Section to heart	Hollande Weigert's Iron Haematoxylin Heidenhain's Azocarmine-Aniline blue	Heart complete
34.	210. mm.	10% Formol	Transverse Section to heart	Hollande Various stains	Every 20th section Intermediate sections
35.	250. mm.	10% Formol	Coronal to heart	Hollande	Inter-vent. septum complete
36.	280. mm.	10% Formol	Transverse Section to heart	Various stains	Interrupted series
37.	310. mm.	10% Formol		Various stains	Selected Blocks
38.	325. mm.	10% Formol	Coronal to heart	Various stains	Intermediate sections
39.	326. mm.	10% Formol	Selected Blocks	Various stains	Intermediate sections
40.	332. mm.	10% Formol	Selected Blocks	Various stains	Intermediate sections
41.	335. mm.	10% Formol	Selected Blocks	Various stains	Intermediate sections

The 6 adult hearts were fixed in Susa, Bouin, Formol-Acetic-Alcohol or in Formol. When the tissue was fixed, blocks were cut from the upper part of the inter-ventricular septum, the entrance of the superior vena cava, the right and left parts of the atrio-ventricular ring and from the atrial and ventricular musculatures.

The precautions which must be taken before any measurements of myocardial cell-size are given, have been well summarised by Ashley (1945). In view of the nature of this investigation, these precautions and methods could not be employed, hence no actual measurements of the sizes of the muscle cells can be given in this work.

No attempt was made to correlate the age of the embryo with its crown-rump length. The errors which are associated with this estimation may be listed:-

(i) Breed of sheep

(ii) Single or multiple pregnancy

(iii) Maternal constitutional factors affecting intra-uterine growth

(iv) Change in length which occurs due to fixation

(v) Degree of flexion of the young embryo.

In view of these numerous factors it was decided to compare the development of the conducting system, solely, with the growth in length of the foetus.

OBSERVATIONS

Whereas the subsequent account of the appearance of the conducting system will be described in the sequence which occurs during its development, the investigation was carried out in the reverse order. Only in this way, can the slight cytological differences which characterise the early stages be related to the well-defined structures of late foetal life.

The criteria, which were adopted before a structure seen in an early embryo was described as a part of the conducting system, were:-

(i) Definite cytological differentiation.

(ii) Constant presence in the same position in all subsequent stages of development.

(iii) Wherever possible, a traceable connexion through differentiated elements to a definite portion of the conduction system.

SINU-ATRIAL NODE

As the sinus venosus is absorbed into the wall of the right atrium, a thickening of the junction appears at its cephalic end. This undifferentiated thickening can be observed in the 9.1 mm. and in the 9.7 mm. embryos. In these embryos the fusion of the thick venous valves/

valves to form the septum spurium can also be seen; however, the undifferentiated thickening of the wall would appear to be a definite entity in relation to, but not due to, these valvular folds.

This thickening of the wall is very soon differentiated, because in the 10.6 mm. specimen (Figures 1 and 2) its cells have a clearer cytoplasm than the atrial muscle cells, and a more spherical and more chromatic nucleus. This change in histological structure is perceptible in the slightly older embryos, and can be readily seen in the 11.7 mm. specimen (Figure 3). As the 9.1 mm. embryo referred to above and this 11.7 embryo were both cut in the coronal plane, it is interesting to compare these series; the differentiated cells can only be made out in the older embryo although the thickening of the wall is present in both embryos.

The cells of the early sinu-atrial node are seen to be paler than the surrounding myocardium, but it is the closely packed mass of spherical, dark staining nuclei which is the most definite guide (Figures 4, 5, 6 and 7). The slight histological changes, mentioned above, are the only cellular differentiation which is present throughout the greater part of foetal life. In the 28 mm. specimen these changes are seen to be very similar but slightly more pronounced. As the atrial muscle becomes more definitely formed with marked longitudinal myofibrils, the paucity of the myofibrils in the nodal cells becomes obvious. Similarly with the increase in size of the atrial muscle-cell their ovoid/

ovoid, vesicular nuclei are spaced further apart, and the compact group of nuclei of the nodal cells becomes more striking (Figures 8, 9, 10 and 11).

In the foetus of more than 100 mm. Crown Rump Length the cells of the sinu-atrial node are separated into a closely packed reticulum, by the invasion of a large number of loose fibrous tissue cells. While in the earlier stages the profusion of nuclei in the node gives the impression that the cells are smaller than the surrounding musculature; it is not until the component cells of the node are separated by this fibrous tissue, that it is possible to state that they have a smaller diameter than the atrial muscle cell.

The relation of neuroblasts to the node can be seen in all the specimens of more than 40 mm. Crown Rump Length, this feature is demonstrated in Figures 12 and 13. In all the subsequent stages the profusion of nerve cells and nerve fibres in and around the node, form one of its most characteristic features (Figures 14, 15, 18, 19 and 20).

The nodal cells join the cells of the atrial wall without any intervention by fibrous tissue or by Purkinje type cells. Although from their inception the cells of the sinu-atrial node are merely a part of the cardiac syncytium, the transition to normal atrial muscle is quite abrupt. Hence/

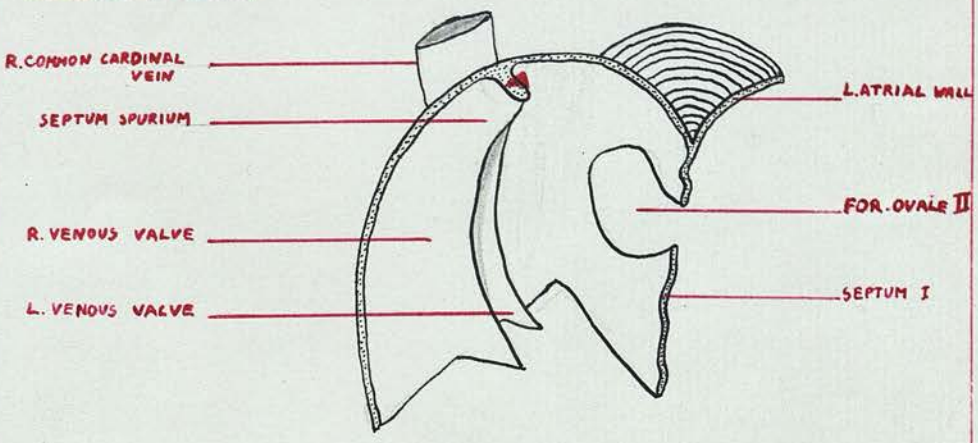
Hence, as early as 11.6 mm. it is possible to describe the gross anatomy of this sinu-atrial node; it is conical in shape with its base to the left of the opening of the right common cardinal vein and its apex projecting up to the sulcus between the vein and the right atrium. From its first appearance the node is precisely at the junction of the atrium and sinus venosus, and there is no evidence to indicate from which structure it is derived.

The rate of spread of this differentiated region is commensurate with the rate of growth of the heart, so that its topography does not change until the 28 mm. stage is reached. The node then begins to extend to the right, ventral to the superior vena cava (right common cardinal vein). In consequence, section of the 28 mm. embryo can show the nodal tissue cut in two places, through the apex of the cone on the right and through the base of the cone on the left (Figure 8). In this manner, the plane of section can make the node appear a double structure, but the two parts are always joined by differentiated cells and it is never truly a double structure.

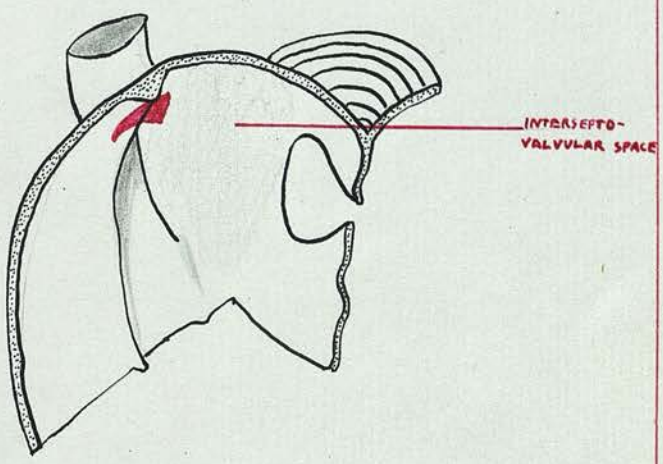
This extension of the node to the right and then caudally in the base of the right venous valve becomes a prominent feature of the sinu-atrial node; it can be seen, for example, at 155 mm. (Figure 15) and in all the older series. By the 210 mm. stage this prolongation can be traced/

Text-figure (2) - A diagrammatic representation of the position and extent of the sinu-atrial node.

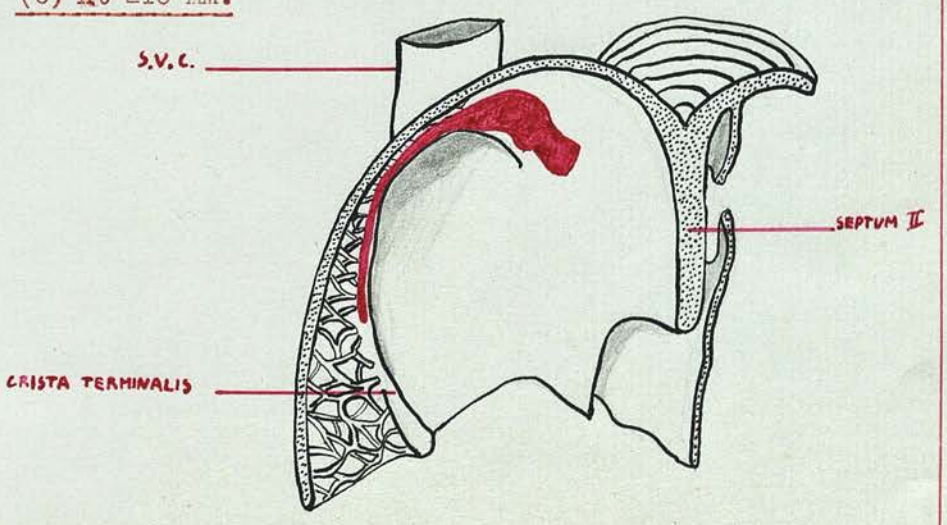
(a) At 11.6 mm.



(b) At 41.9 mm.



(c) At 210 mm.



The sinu-atrial node, drawn in red, is shown as it would appear if the atrial wall was transparent.

Text-figure (3) - Approximate scale drawings of the outlines of the sinu-atrial node.

At 11.6 mm.

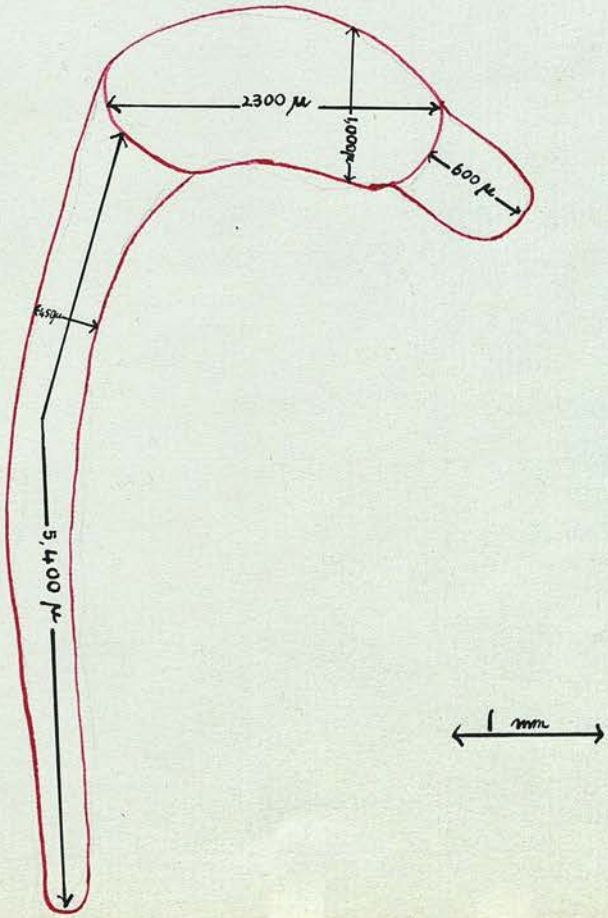
250 μ x 100 μ

At 41.9 mm.

700 μ x 360 μ

Atrial Wall-------- 30 μ thick
Common Cardinal V.- 10 μ thick

At 210 mm.



traced for a considerable distance down the crista terminalis (base of right venous valve); but neither at this stage, nor at any other stage of development, can a specialized connexion with the atrio-ventricular node be observed.

In the earlier stages the base of the conical sinu-atrial node is related to the left venous valve and hence to the interseptovalvular space. When, about the 70 mm. stage, the septum secundum is formed as an infolding of the atrial wall in the interseptovalvular space, the left edge of the sinu-atrial node is drawn into the dorsal and cephalic part of the septum secundum. Text-figure (2) is intended to show in diagrammatic form the changes which are taking place in the shape of the sinu-atrial node during the absorption of the sinus venosus and during the formation of the septum secundum. The great increase in size of the sinu-atrial node during pre-natal life can be appreciated from Text-figure (3), although it is not claimed that the measurements given are anything more than gross approximations.

As the result of these processes, the sinu-atrial node of the late foetus is kidney shaped, with the concavity of the kidney resting in the left part of the sulcus between the superior vena cava and the right atrium. A long slender process extends from the right edge of the node caudally in the crista terminalis, while from the left edge of the node, a flattened band projects into the adjacent part of the septum secundum.

The/

The blood supply of the sinu-atrial node.

The first sign of a blood vessel in the node is seen in the 16.6 mm. embryo, a small vein can be seen leaving the nodal tissue and entering the common cardinal vein. The well-defined artery of the sinu-atrial node is not seen, however, until the 25 mm. stage (Figures 6 and 7). The origin and course of this artery can be traced with ease in the 44 mm. specimen, which is cut in the sagittal plane; it arises near the origin of the right coronary artery and courses up the ventral wall of the atria, dorsal to the aorta. On the cephalic wall of the right atrium it divides into two branches, the smaller left branch runs to the left side of the opening of the superior vena cava, the right branch runs caudally in the crista terminalis, tunnelling through the right prolongation of nodal tissue. The artery can be seen in Figures 9, 10, 11, 12, 13, 14 and 15. The presence of nodal tissue cells in the tunica media of the nodal artery was not observed in the present preparations.

The openings of the pulmonary veins.

These regions were scrutinised in all the preparations, but at no stage of development was any specialized tissue seen in relation to these openings. Nerve cells and ganglia can, however, be demonstrated in the epicardium adjacent to the opening of the pulmonary veins.

ATRIO-VENTRICULAR CONDUCTION SYSTEM.

In the 5.4 mm. embryo there is a low ridge which represents the muscular interventricular septum, and there is no sign of any specialized muscle tissue. The atrio-ventricular muscular connexions are complete, but there is a tendency for the muscle fibres of the atria and ventricles to run parallel to each other in the wall of the atrial canal. (Figure 38).

As soon as the interventricular septum is well-developed, 6.5 mm. and 6.7 mm. (Figures 21 and 22), a different type of cell can be seen dorsal to the dorsal endocardial cushion, and immediately cephalic to the point where the muscular interventricular septum reaches the dorsal endocardial cushion. These cells have well-marked cell membranes, completely pale cytoplasm and ovoid dark nuclei. Strands of these pale cells run along the upper border of the muscular interventricular septum. These strands have thin lateral connexions, but these connexions do not confuse the main direction of the strands,(Figure 25). These cellular strands can be traced up to the caudal end of the inter-atrial septum, at the point where the right venous valve is attached, and hence in relation to the left horn of the sinus venosus. At their atrial end these cells can be seen to connect, without any intervening specialized structure, with the atrial muscle cells, for examples 6.7 mm., 10.6 mm. (Figures 28, 29 and 30). At their ventricular end these strands/

strands are continuous with the muscle of the ventricular septum, 6.7 mm. (Figures 21 and 22) and 11.6 mm. (Figure 31). This structure described above has been interpreted as the first sign of the atrio-ventricular bundle.

There is no specialized structure at the atrial end of this early atrio-ventricular bundle, but very shortly the cells at its upper end appear dark staining and smaller than the cells of the bundle. These dark cells are seen, for example, in the 8.4 mm. (Figures 23 and 24), 9.7 mm., 11.7 mm. and 11.6 mm (Figure 31). These dark cells are arranged in a nodule at the atrial end of the bundle. The time of appearance of this nodule appears slightly variable when compared with the Crown-Rump Length, because although it is present in the embryos mentioned above it is absent in the 9.1 mm. (Figures 26 and 27) and in the 10.6 mm. (Figures 28, 29 and 30). The presence of this nodule is constant after 11 mm., and it has been interpreted as the first sign of the atrio-ventricular node.

Atrio-ventricular Node.

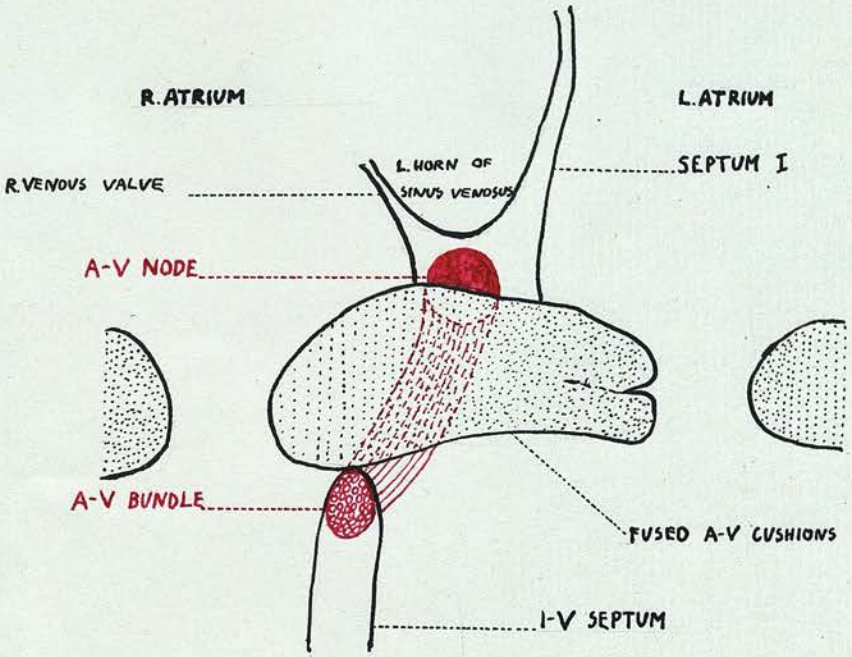
The early atrio-ventricular node can be described as a mass of cells, whose base rests on the ventral surface of the left horn of the sinus venosus. From its caudal surface the pale, loose cells of the bundle pass immediately on to the crest of the muscular inter-ventricular septum/

septum, dorsal to the dorsal endocardial cushion. The cells of the node have poorly marked cell boundaries, their cytoplasm staining darker than the atrial muscle cells and much darker than the cells of the bundle. The nuclei of the cells of the atrio-ventricular node are very similar to the nuclei of the bundle, very dark staining and ovoid.

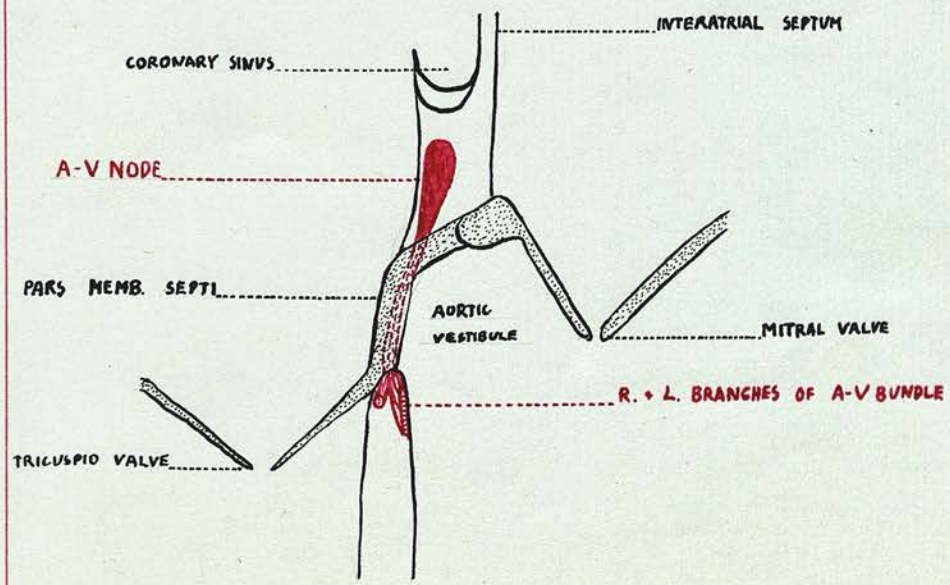
The subsequent development of the atrio-ventricular node resembles that of the sinu-atrial node; the node becomes more apparent, more by changes in the surrounding atrial muscle cells than by any change in the node itself. The cell membranes of the atrio-ventricular node become more prominent at an earlier stage of development than those of the sinu-atrial node; in the atrio-ventricular node the cellular membranes are definitely recognisable at the 25 mm. stage (Figures 41 and 42). In the same way as in the sinu-atrial node, the cells of this node are abruptly continuous with the cells of the atrial walls without the intervention of any specialized structure. The atrial muscle cells, in the region of the node do form a network but the cells of the network are similar in every way to the cells of the atrial walls. The invasion of the node by fibrous tissue occurs at the same time as in the sinu-atrial node but it does not reach the same extent in the atrio-ventricular node.

Text-figure (4) - A schematic representation of the relation between the A-V bundle and the pars membranacea septi.

(a) In the early embryo



(b) In the definitive state



There are small blood vessels clearly seen in the atrio-ventricular node at 20.1 mm. (Figures 35 and 36), and throughout pre-natal life the node is highly vascularised. The blood supply is administered by a mass of capillaries with their associated arterioles and venules. (Figures 39, 40, 45, 46, 49, 50 and 56). There is no special 'artery to the node'. A few small blood vessels pass from the node down into the common trunk of the bundle. (Figure 40).

The nervous tissue appears in relation to the atrio-ventricular node about the 40 mm. stage. A group of neuroblasts can be seen in the periphery of the node in the 44 mm. specimen (Figures 49 and 50). In the later specimens the nervous ganglia are very abundant and large nerve trunks can be traced through and around the node into the common trunk of the bundle. (Figures 51, 52, 53 and 54).

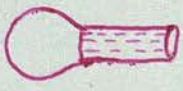
The gross anatomy of the atrio-ventricular node of the early embryo has been described above as a mass of cells in a nodule, which does, however, soon become flask-shaped. The early node lies symmetrically between the right and left atrio-ventricular openings, ventral to the left horn of the sinus venosus and immediately dorsal to the dorsal endocardial cushion. Due to the separate attachments of the inter-ventricular and interatrial septa to the septum intermedium, and to the longitudinal division of the truncus arteriosus, the lateral relations of the node become the endocardium of the right atrium and the right dorsal sinus of the aortic valve respectively. Text-Figure (4).

Text-Figure (5). - Approximate projection drawings of the atrio-ventricular node. Magnification X 40.

At 11.7 mm.

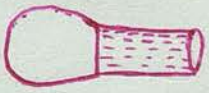
180 μ x 130 μ x 120 μ

At 22.8 mm.



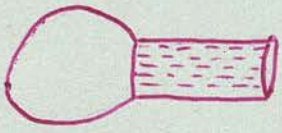
200 μ x 160 μ x 150 μ

At 44 mm.



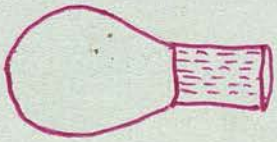
280 μ x 230 μ x 200 μ

At 92 mm.



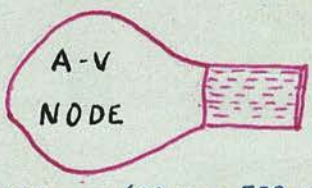
500 μ x 400 μ x 350 μ

At 155 mm.

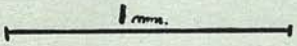


600 μ x 450 μ x 350 μ

At 210 mm.



800 μ x 600 μ x 500 μ



The increase in size of the atrio-ventricular node can be appreciated by reference to Text-Figure (5), and it will be appreciated that this increase in actual size means a very marked reduction in relative size.

The shape of the node remains flask-shaped with the mouth opening caudally and ventrally to be continuous with the common trunk of the bundle. There are no prominent, specialized prolongations from the atrio-ventricular node. But in the 25 mm. and the 44 mm. specimens which are cut in the sagittal plane, it can be observed that the cephalic surface of the node is indented by a fibrous band which proceeds from the aortic valves to the cephalic surface of the coronary sinus. This band limits a blunt process which points towards the ventral wall of the left atrium. In the sections cited above this portion of the node can be seen as separate from the rest of the atrio-ventricular node.

Common trunk of the atrio-ventricular bundle.

As has been described above the appearance of the common trunk of the bundle precedes that of the atrio-ventricular node; and it is therefore the first portion of the conducting system to present itself.

The site of the early differentiated cells of the atrio-ventricular bundle permits of different interpretations as to their origin. The possible sites of origin are the/

the caudal end of the dorsal wall of the atrium, the wall of the atrial canal and the portion of the interventricular septum which runs up to the dorsal endocardial cushion. From observation particularly of the 6.5 mm. and 6.7 mm. embryos, it can be seen that there is no barrier between these cells and the cells of the posterior wall of the atrial canal, which are in immediate relation, and it would appear that they had been derived from the wall of this canal. But it is stressed that owing to the primitive state of the heart chambers and of the myocardium, with the gradual blending of the one cavity into the other, it is impossible to be dogmatic regarding the origin of the cells of the common trunk.

The histological structure of the early bundle cells is found to be that of a loose syncytial network with circular or oval dark nuclei and colourless cytoplasm. There were no myofibrils or other intracellular structures, when at the same stage of development the myofibrils were clearly seen in the ventricular myocardium. By the 20 mm. stage the loose network has become condensed and the lateral connexions of the strands are less prominent, for example 20.1 mm. (Figures 35 and 36) and 22.8 mm. (Figures 39 and 40). So the common trunk is formed by a group of pale cellular strands, the nuclei are less chromatic but they have become more definitely spherical. The interventricular foramen closes between

the 15 mm. and the 20 mm. stage, and the development of this definite compact bundle immediately succeeds this event. Also around the 20 mm. stage there can be seen between the bundle cells and the normal ventricular myocardium, some flattened cells with long narrow nuclei; these cells are the first sign of the fibrous tissue sheath of the bundle. With the advancing differentiation of the fibrous tissue throughout pre-natal life, this fibrous tissue sheath becomes correspondingly well-defined. Longitudinal myofibrils appear in the common trunk of the bundle about 100 mm. and the cytoplasm acquires fine granules. But these intracellular inclusions do not affect the region around the nucleus which remains clear. The nucleus has become less chromatic and is vesicular. Double nuclei are rarely seen at any stage in the common trunk but they can be found between the 100 and 210 mm. stages. There are no marked changes between the cell of the late foetal common trunk and the adult cell, the myofibrils of the latter are arranged in the periphery and the cellular diameters are greatly increased. The cell of the adult common trunk is a typical Purkinje cell.

In the second half of pre-natal life the strands of cells in the common trunk are separated from each other by fibrous tissue. Amongst this fibrous tissue support, in/

in the 138 mm. specimen, nerve trunks can be traced down amongst the Purkinje cells. In the older specimens there are numerous large nerve trunks accompanying the bundle. From an earlier stage, namely 20 mm., a large number of small blood vessels can be seen amongst the specialized cells. From the examination of the ordinary microscopic preparations it would appear that the common trunk is as well supplied with blood vessels as the ventricular myocardium.

The pre-natal changes in the common trunk as a whole are those of lengthening and relative attenuation. The 11.7 mm. bundle is 350 μ in length and the diameter is 100 μ, whereas the common trunk of the 210 mm. has lengthened to 2,500 μ the diameter has only increased to 200 μ.

The changes in the course and relations of the common trunk are consequential to the changes in the developing heart and are schematically represented in Text-figure (4). The course of the late foetal A-V bundle is from the node in a ventral direction and it tunnels through the right part of the fibrous trigone of the heart. At its origin it is separated by some normal atrial muscle from the endocardium of the right atrium, then it lies in relation to the attachment of the medial cusp of the tricuspid valve. At the ventral limit of this attachment the trunk divides into its/

its two branches. Throughout its course the common trunk has a slight ventral convexity. The common trunk does not give off any branches apart from the two terminal branches. (Figures 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 58).

Left Branch of the Atrio-ventricular Bundle.

The ventral end of the common trunk of the bundle is originally continuous with the ordinary ventricular muscle cells, this can be seen at 11.6 mm. (Figure 31). But by the 14.7 mm. stage the differentiated cells can be traced into a definite fasciculus which runs to the endocardium of the left ventricle, and then into a trabecula which lies free in the left ventricle close to the inter-ventricular septum. The differentiated cells can be recognised in the 14.7 mm. specimen as far as the mid-point between the bifurcation of the common trunk and the apex of the left ventricle. There is a gradual transition from the large, pale, conducting tissue cells, with their dark spherical nuclei, to the smaller, more eosinophilic, ventricular myocardial cell with ovoid, vesicular nuclei. This same phenomenon can be observed in the 18.4 mm. embryo (Figures 32, 33 and 34). But in this slightly older specimen the differentiation can be traced still nearer to the apex. Owing to the gradual nature of the transition with the numerous intermediate stages it is difficult to demonstrate this/

this phenomenon by photomicrography. A low-power view which can show the typical conducting tissue cell on the one hand and typical ventricular muscle on the other, cannot have sufficient magnification to show cellular detail. Correspondingly a high-power photograph can only show a limited portion of the transition. However, a careful study of the serial sections leaves no doubt that this transition is quite real.

When the 32.2 mm. stage is reached the differentiated cells can be traced right down to the small fasciculi which traverse the cavity of the left ventricle near to its apex. These small fasciculi blend into the ventricular myocardium in relation to the papillary muscles. In the later specimens the left branch divides into two trabeculae, which are associated with the two papillary muscles of the left ventricle.

From the 30 mm. stage onwards the histological changes in the differentiated cells of the left branch are the same as have been described above as occurring in the common trunk of the bundle. The constituent cell of the definitive left branch of the bundle is the typical Purkinje cell.

As the size of the ventricles and the thickness of the interventricular septum increase, the left branch is gradually/

gradually absorbed under the endocardium of the left side of the interventricular septum. This absorption proceeds from above downwards, but it never quite reaches the apex, so that the distal end of the left branch in the late foetus is still lying free in the cavity of the ventricle. Throughout its course under the endocardium numerous branches from the left branch can be traced into the interventricular septum. This latter point is well demonstrated in the 250 mm. specimen.

The presence of a definite fibrous tissue sheath for the left branch can be seen first at about 40 mm. As no special neurological staining method was employed, it was not possible to show any nerves in association with this branch until 210 mm. when the neurolemmal and fibrous tissue cells of the nerve trunks could be demonstrated. There is no large or special blood vessel to the left branch, it is supplied to the same extent and in the same manner as the surrounding myocardium.

Right Branch of the Atrio-ventricular bundle.

The differentiation of the right branch takes place in the same general manner as occurs in the left branch. But due to the fact that the right branch is a compact rounded bundle, the changes can be seen with greater ease than in the more diffuse left branch. The right branch cannot be definitely/

definitely distinguished until the 16.6 mm. stage. Although it will be appreciated that, in such a gradual differentiation, the stage at which the parts of the system are stated to be apparent can only be approximate.

By the 20.1 mm. stage the right branch can be traced into the trabecula which is the anlage of the moderator band. In this band it can be seen that the undifferentiated muscle of the band is arranged in a compact bundle for some distance below the region of transition from the conducting tissue cells (Figures 35, 36 and 37). A comparison of the undifferentiated cells of the right branch at 20.1 mm. (Figure 37) with the definite differentiation well down the left branch in the 18.4 mm. embryo (Figures 32 and 33), demonstrates the fact that the right branch is differentiated later than the left.

The broader paler cells of this branch can be traced farther and farther along the moderator band as the age of the embryo increases. Thus at the 44 mm. stage they can be traced almost to the free wall of the ventricle (Figure 59). By the 63 mm. specimen (Figure 60) the branch lying amongst the ordinary myocardium of the moderator band is quite distinct. The fibrous sheath of the right branch can also be seen in this illustration (Figure 60); it is not possible to be definite about its presence before this stage.

The histological structure of the right branch undergoes the same changes as are indicated above for the left branch and the common trunk. These changes are also shown in Figures 60, 61, 62, 63 and 64. The presence of double nuclei is most noticeable in the late foetal stages.

The topography of the right branch is interesting not only for its own sake but also for the information which it provides on the development of the right ventricle. The bifurcation of the common trunk occurs at the ventral limit of the medial cusp of the tricuspid valve, that is the cusp formed from the fused atrio-ventricular cushions. So that the point of origin of the right branch is where the muscular interventricular septum meets the parts of the pars membranacea septi, which are formed from the atrio-ventricular cushions and from the bulbar ridges. From the junction of these three formations the infundibulo-ventricular crest, formed from the right bulbar ridge, sweeps across the ventral aspect of the tricuspid valve to reach the free wall of the right ventricle. In consequence the right branch of the bundle can be found immediately caudal to the attachment of the infundibulo-ventricular crest to the interventricular septum. There is a remarkably constant but small papillary muscle, whose chordae tendinae run to the junction of the medial/

medial and ventral cusps of the tricuspid valve. This papillary muscle leaves the septum just caudal to the attachment of the infundibulo-ventricular crest, but the right branch of the bundle passes, under the endocardium, between the crest and the papillary muscle.

As in the case of the left branch, the right branch is originally completely free in the cavity of the ventricle, but the proximal portion is gradually absorbed into the septum. So that the point where the moderator band leaves the septum becomes progressively nearer the apex. The right branch was not seen to give any branches until it reached the free wall of the right ventricle. There is a large artery in the substance of the moderator band which sends branches to the right branch of the bundle. This artery is a branch of the ventral interventricular branch of the left coronary.

Subendocardial Purkinje Network.

At 71 mm. some larger cells, which have spherical nuclei, can be seen immediately under the endocardium of both ventricles (Figure 65). A small false tendon containing these cells can be seen to reach the endocardium over a papillary muscle and the enclosed fibres open out under the endocardium.

But it is in the 92 mm. specimen that these cells can be distinguished with confidence from the ordinary myocardium/

myocardium. The Purkinje fibres are seen to be larger and they have a spherical nucleus, their intercellular boundaries are very distinct. The presence of myofibrils in the Purkinje fibres can be recognised in the 138 mm. and 155 mm. series; and between these stages and the 280 mm. specimen the Purkinje fibres stain darkly due to the presence of these myofibrils and of intracellular granules. In this period the most characteristic feature of the fibres is the peri-nuclear clear region. In these later foetal stages the presence of double nuclei in the Purkinje fibres is much more frequent.

As soon as the Purkinje fibres can be clearly recognised under the endocardium, at around the 100 mm. stage, they can be traced to transitions to normal myocardial cells. The larger subendocardial fasciculi, of the late foetus, are surrounded by a thin but definite fibrous tissue sheath.

Myocardial Purkinje Network.

The presence of Purkinje cells in the depth of the myocardium of the ventricles, can be seen in the 155 mm. specimen. In all subsequent stages this network is very profuse, and invades every portion of the ventricular myocardium at 210 mm. (Figure 66). Their histological characteristics are the same as those of the subendocardial network, but the fibrous/

fibrous tissue sheath cannot be seen around the smaller fasciculi. The gradual transition to the ventricular myocardial cells can be seen in all these specimens.

In the complete serial sections of the inter-ventricular septum of the 250 mm. heart, an attempt was made to trace the differentiated Purkinje cells from the endocardium on the one side of the septum to the endocardium on the other side. But in each bundle, which was followed, the Purkinje cells were gradually transformed into an ordinary ventricular myocardial cell; and it was impossible to establish a specialized muscular connexion between the terminal ramifications of the main branches of the atrio-ventricular bundle.

Atrio-ventricular Muscular Continuity.

The existence of a continuity across the atrio-ventricular junction, apart from the atrio-ventricular bundle, was looked for in all the series. It can be stated that at no stage of development is there any specialized muscular continuity, apart from the common trunk of the atrio-ventricular bundle.

The interruption of the complete unspecialized muscular continuity which exists in the early embryo is a gradual/

gradual process. This process can be divided, to some extent artificially, into four stages:-

(i) Obvious complete continuity - up to 5.4 mm. (Figure 38).

(ii) The formation of the atrio-ventricular valves causes some attenuation of these connecting muscular strands - from 6.5 mm. to 10.6 mm. (Figures 21, 22, 29 and 30).

(iii) The invasion by young fibroblastic tissue makes it likely that atrio-ventricular continuity has been severed - from 11.6 mm. to 29.7 mm.

(iv) These fibroblasts have laid down a collagenous ring which can be demonstrated by histological technique - in all the specimens of 32.2 mm. and longer.

MODE OF DIVISION OF THE CONDUCTING TISSUE CELLS.

In spite of the large number of preparations which have been examined in the course of this work, a mitotic division was not seen in any cell which was undoubtedly a portion of the conducting system. This absence is more striking in the earlier stages when there is so much active mitosis among the other embryonic tissues, including the ordinary myocardium.

The possibility of an amitotic method of cell division was suggested by the presence of the cells with double nuclei. But the intermediate stages of an amitotic method of division, were not definitely observed. Superimposition of the two closely related nuclei can often give the false impression of a 'dumb-bell' shape to the nucleus (Figure 62).

Purkinje cells with double nuclei are a characteristic feature of the late foetus (Figures 61, 62 and 66). They are not commonly encountered in the earlier stages (Figures 59, 60 and 65), and they are less common in the adult Purkinje cell (Figure 64). They are more frequent in the peripheral ramifications than in the common trunk, compare Figure 58 with Figure 66. They are not a feature of the sinu-atrial or atrio-ventricular nodes at any stage of their development. But due to the compact, syncytial nature of the early nodes, it is not possible to be certain of the presence or absence of double nuclei, when the cell membranes are so ill-defined.

DISCUSSION/

DISCUSSION

In the course of this discussion the results of this present work will be correlated with those of previous workers. However, the greatest importance should be attached to the comparison with the results of the two previous workers on the sheep embryos, namely Sanabria (1936) and Field (1951a). The definitive structure of the conducting system varies so strikingly from species to species, that only the most general comparisons should be made with the development in other species.

SINU-ATRIAL NODE.

Due probably to differences of interpretation, the age at which the node becomes apparent has varied with different observers.

In the sheep, Sanabria was able to see differentiation of the nodal cells at 10 mm., and he had seen thickening of the auricular myocardium in the region of the sulcus terminalis from the 7 mm. stage. On the other hand Field was unable to describe the advent of this node until about the 100 mm. stage, and he asserted that the differentiation described by Sanabria is apparent under low magnifications only. But Sanabria's photograph (Figure 18, Plate III, x 72) shows some differentiation at 10 mm. and a definite/

definite node at 70 mm. (Figure 23, Plate III, x 112). There can be no doubt that the tissue described and illustrated by Sanabria, is identical to that which has been interpreted as the anlage of the sinu-atrial node in this present work; it is hoped that the figures of this thesis show the presence and nature of this early differentiation.

The fact that these cells can be found constantly in the precise position of the adult sinu-atrial node, was regarded as sufficient evidence to indicate that the node appears at 10 mm. in the sheep embryo. Field, p.139, asserts that it is only because one is "armed with the knowledge that the node will develop in the sulcus terminalis, one may interpret the mass of cells at the base of the right venous valve as its primordium". But reference to older stages is a valuable aid in an embryological investigation and these altered cells are the primordium of the sinu-atrial node.

Physiological evidence would support the early origin of the sinu-atrial node. Patten and Kramer (1933) have shown, in the chick, that a rhythmic heart beat is established before the paired heart tubes have even fused at their caudal ends to form a sinus venosus, and Goss (1942) was able to observe, in a tissue culture preparation of a rat embryo, the atria of the paired heart tubes acting as pacemakers/

pacemakers at the 7 somite stage. So there must be a mechanism for the initiation of the heart beat before the earliest differentiation of the sinu-atrial node.

Steinon (1926) could recognise the human sinu-atrial node at 48 mm. In his series of human embryos, Sanabria (1936) was able to indicate a localised thickening of the myocardium at 11 mm. and a definite node with differentiated cells at 19 mm. Similar conclusions were reached by Walls (1947) who observed at 10 mm. a compact cell group with a stronger chromatin reaction than the surrounding myocardium.

Shaner (1929) working on a series of calf embryos could not describe the presence of the node until 100 mm.

The origin of the sinu-atrial node was regarded by Keith and Flack (1907) to be a reduced portion of the sinu-atrial ring. This opinion was arrived at from comparative evidence, but to all the embryological investigators the node appears as a new development, appearing and differentiating in a definite manner. Shaner (1929) found that in the calf the node developed in the ventral wall of the common cardinal vein and only sank into the sulcus terminalis/

terminalis at birth. This observation was not confirmed in the present series of sheep embryos, the first sign of the node at 10 mm. being topographically identical to the position of the developed structure of the adult. Field agrees with Sanabria in stressing the difficulty in stating whether the structure is derived from the right atrium or from the sinus venosus. The opinion of the author is that the first appearance is at the junction of the sinus venosus and the right atrium, and there is no embryological evidence to indicate from which structure it is derived.

Although Pace had described a duplication of the sinu-atrial node in the sheep, he rescinded this view in his review of the conducting system in 1924; but he stressed that two extensions run out from the node, one into the interatrial septum and the other into the crista terminalis. Segre (1926) who studied four human hearts reached the conclusion that the node was double in the foetus and that the two portions joined in the adult. At no stage in the present series were two separate nodes observed, although individual sections could often give this impression.

An interatrial node was described by Shaner (1929) in the calf, this node was said to appear at 13.5 mm., increasing in size and differentiating into nodal tissue

until/

until 31.5 mm. when it gradually diminishes and disappears at 90 mm. This separate node as described by Shaner was not found at any stage in the sheep's heart. But the portion of the atrio-ventricular node which projects towards the left atrium can be seen in the sagittal series (25 mm. and 44 mm.) as a separate portion of nodal tissue. Blair and Davies (1935) have offered a similar explanation of Shaner's findings, from their studies on the adult ox heart.

No specialized muscular connexion between the nodes was seen at any of the stages of development which were examined. Thorel (1910) has described a connexion between the nodes, and Tudor Jones (1920) has illustrated this connexion in a Bielchowsky preparation of a 24 mm. human embryo.

Pace (1924) mentions that although he found, in the pig, numerous ganglia around the openings of the pulmonary veins, he did not find any evidence of specialized tissue. The present observations in the sheep would entirely support Pace's opinion; and oppose the view of Glomset & Glomset (1940a) who found tissue similar to the nodal tissue at the opening of the pulmonary veins. Field (1951a) has supported the contention of Glomset & Glomset that the histological structure of the sinu-atrial node is due to its location in the depth of the sulcus, rather/

rather than any definite specialization as a neuro-muscular structure.

In conclusion, it can be stated that in the course of this work, the sinu-atrial node has appeared as a unique and well-defined structure, with most definite histological characteristics. There are a large number of nervous structures in intimate relation to the node, but these nervous structures are found to a less extent in other parts of the atrial walls, not related to any specialized nodal tissue. Further the sinu-atrial node can be recognised before nerve cells can be seen in the heart, using ordinary histological methods. It is suggested that the study of the ontogeny of the sinu-atrial node would tend to accentuate its importance as an anatomical entity.

ATRIO-VENTRICULAR CONDUCTION SYSTEM.

Atrio-ventricular node and bundle.

The previous workers on sheep embryos, Sanabria (1936) and Field (1951a), have reached the conclusion that the atrio-ventricular bundle is the first part of the conducting system to be differentiated. Sanabria identified the common trunk of the bundle at 5 mm. when the syncytial fibres of the posterior wall of the atrial canal become arranged into a network, which has its long axis running from the atrium to the/

the ventricle. Sanabria states that the cytoplasm of the network is vacuolated and its nuclei are more chromatic than in the surrounding myocardium.

Field was able to notice this re-orientation at 4.5 mm. but could not observe cellular differentiation in the bundle until 10 mm. The results reported in this thesis would support these previous works as it was possible to recognise a differentiated atrio-ventricular bundle at 6.5 mm. Although the atrio-ventricular bundle has appeared at this stage the peripheral atrio-ventricular connexions, around the atrial canal, are unmistakably present. These peripheral connexions are not definitely severed until about the 20 mm. stage. This development of a specialized bundle before the destruction of the other connexions has been used by Davies & Francis (1941, 1946) and Davies (1942) as collateral evidence in favour of their theory, that the conducting tissue is a neomorphic development and not a 'remnant' of the more extensive junctional tissues of the lower vertebrates.

The insertion of the interventricular septum into the atrio-ventricular ring, dorsal to the dorsal endocardial cushion, is probably the factor which determines the part of the ring which will become differentiated into the bundle. This is in accordance with the view of Keith & Flack (1907) who describe the/

the presence of the bundle in the part of the heart which was the least disturbed during the course of development. The persistent connexions at the lateral sides of the atrio-ventricular rings, as described by Kent (1893, 1913) were not observed in any of the later stages of the development of the sheep's heart.

The development of a condensed mass of cells at the cephalic end of the atrio-ventricular bundle was seen at 8.4 mm. Sanabria and Field describe the appearance of the node at later stages of development, 20 mm. and 14 mm. respectively. Using the criteria defined at the beginning of this discussion it is possible to describe the node at 8.4 mm., but the author would agree with the previous workers that it is necessary to wait until the 20 mm. stage before a definite nodal structure can be observed.

The atrio-ventricular node, like the sinu-atrial node, is differentiated from the primitive myocardium, and so is from the outset continuous with the myocardium. In the adult sheep, as in the foetus, the fibres of the atrio-ventricular node are continuous on the one hand with the bundle, and on the other with the atrial musculature. So the author must disagree with Glomset & Glomset (1940b.) who could not find any connexion between the node and the atrial musculature. In view of this observation these authors prefer/

prefer to reject the name atrio-ventricular bundle and revert to the name 'His bundle'. But they do admit the presence of the bundle in the ungulates and it is thus surprising that Field (1951a, p.145) should state "The very existence of an A-V bundle in the sheep has recently been roundly denied by Glomset & Glomset (1940)".

The evidence which can be gained from the literature on the origin of the atrio-ventricular node and bundle in other vertebrates is inconclusive. The appearance of the node was described as preceding the bundle by Shaner (1929) in the calf, he recognised the node at 9 mm. and concluded that the bundle arose from the node and appeared at 23 mm. Walls (1947), using human material, supported the findings of Shaner. The evidence which Walls cites in his paper would not appear to be conclusive. He was able to examine two human embryos of 8 mm., in one he found a node and a bundle but in the other he found no trace of the conducting system. Walls's conclusion was based on the fact that the node in his first specimen appeared to show active growth. There would not appear to be any definite cell division in the photograph of this node, Fig.2, Plate 2.

Sanabria (1936, p.52) in his description of the human development described the condition at 6 mm. as

"La/

"La musculature postérieure de ce canal acquiert déjà ses caractères propres : la différenciation fibrillaire y semble moins poussée, les fibres sont plus pâles et affectent une disposition réticulaire typique". The presence of the atrio-ventricular bundle is described, with greater certainty, at 11 mm. in these words "Le canal auriculaire est médian, la musculature de sa paroi posté-rieure, sensiblement différente de celle des régions voisines, donne naissance par son extrémité inférieure à une traînée d'éléments cellulaires qui s'enfonce dans le coussinet endocardique postérieur jusqu'à son bord libre antérieur". Sanabria does not describe the appearance of the human atrio-ventricular node until 27 mm.

In general descriptions of the development of the human heart, Mall (1912) and Waterston (1918) refer to the development of the atrio-ventricular conducting apparatus. These two authors stress the importance of the 'undermining' of the atrio-ventricular sulcus in the formation of the cusps of the mitral and tricuspid valves. They regarded the atrio-ventricular bundle as being derived from the remaining part of the atrio-ventricular ring, and they did not discuss the appearance of the atrio-ventricular node. The deduction can be made from the observations in this thesis, that at around the 15 mm. stage when the interventricular foramen has closed, the atrio-ventricular bundle becomes a very distinct/

distinct and obvious structure. This time relationship was also reported by Field (1951a) but it was very clearly expressed by Waterston (1918, p.300) in these words, "It may here be pointed out that the process of development of the heart shows a short-circuiting of part of the blood stream. In the early stages the blood passes from the atrium to the ventricle and thence to the bulbus cordis. The alteration in the condition of the atrial canal allows part of the blood stream to pass from the right division of the atrium into the bulbus cordis and so to the truncus arteriosus, without passing through the primitive ventricle at all. At the stage when the right and left ventricles become completely closed off from one another, this short-circuiting of part of the blood stream necessitates an alteration in the original peristaltic wave, so that the right and left ventricles may beat simultaneously, and it is when this separation has been effected that in my specimens there is the first indication of the presence of the atrio-ventricular bundle". This accurate summary does have the additional merit that it can associate the structure of the system with its functional importance.

The/

The Main and Peripheral branches of the Atrio-ventricular Bundle.

The right and left branches, which arise from the ventral limit of the common trunk, are progressively differentiated from above, the left in advance of the right. The earlier appearance of the left branch was mentioned by Mall (1912), and was clearly detailed by Walls (1947). In view of the ultimate predominance of the left ventricle over the right, this fact could be used as evidence in favour of the functional importance of the conducting system.

Sanabria (1936, p.65) describes the differentiation of the two main branches as occurring "par un phénomène d'induction" as soon as the upper end of the ventricular trabeculae fuse with the common trunk. Field(1951a) describes the differentiation as occurring 'in situ' and does in the course of his observations describe the order in which the portions of the conducting tissue make their appearance. The theory which would explain all the findings in this investigation, would be that a wave of differentiation spreads down into the ventricles from the differentiated cells of the common trunk. At any stage of development, the transition between specialized muscle and normal myocardium can be observed. At 10 mm. this transition will occur on the crest of the interventricular septum, at 40 mm. it occurs in the length/

length of the moderator band, at 100 mm. it occurs under the endocardium, and in the late foetus and adult the transition occurs in the depths of the ventricular myocardium. The observation, made in this work, that the myocardial cells immediately in advance of the differentiated cells become arranged into cylindrical trabeculae, in preparation for differentiation, must be regarded as important. There would not appear to be any mention of this fact in the literature, and yet it is evidence that the conducting tissue is formed by differentiation of the ordinary myocardium.

The absence of any definite mode of cell-division in the Purkinje fibres can be used as evidence in favour of this theory. If the conducting system is formed from the normal myocardium then there is no need for the cells of the system to divide since it can expand by engulfing further myocardial cells as the differentiation proceeds. A reasonable postulate would be that, the Purkinje cell is so specialized for a particular purpose that, like a nerve cell, if cannot undergo cell division once it is fully formed. This theory is to some extent the reverse of Retzer's (1920) who saw in the conducting system the site of regeneration of the cardiac muscle; bearing the same relation to the cardiac muscle as the basal layer of a stratified squamous epithelium bears/

bears to the more superficial layers. This theory of Retzer was strongly challenged by Cady (1921) and Tufts (1921), and the author would disagree with it on the grounds that the Purkinje fibres do not show active cell division.

While Field calls attention to the fact that the right branch is at first free in the cavity of the right ventricle, and is subsequently partially absorbed into the septum (Figure 23a and b); he shows the left branch as lying in the septum from the outset. The author would extend Field's observation and states that the left branch also is originally free and in the adult the upper two-thirds are absorbed into the septum.

The Nervous Tissue of the Conducting System.

This normal embryological study of the conducting system does not demonstrate selectively the nervous elements of the system. Using non-specific staining methods, a histologically differentiated conducting system can be observed before the nervous elements can be demonstrated. Neuroblasts are found in relation to the nodes at about the 40 mm. stage, and in all the later stages large numbers of nerve-cells and nerve trunks can be found in relation to the entire system. The presence of nerve fibres in the atrio-ventricular bundle/

bundle was described by Tawara (1906) and subsequent accounts of the nervous tissue of the conducting system have been furnished by Wilson (1909), Morrison (1912), Meiklejohn (1913) Woollard (1926), Blair & Davies (1935), Monidez (1943), McMahon & Stotler (1947), Field (1951b) and Landau (1951). In view of these reports and the present findings it can be stated that the conducting system should be regarded as a neuromyocardial system. Any functional interpretation of the conducting system must contain references to this important nervous element.

The Fibrous Tissue Sheath of the Atrio-Ventricular Bundle.

Fibroblasts can be recognised in the heart of embryos of 15 - 20 mm., and at this early stage a tenuous fibrous tissue sheath can be seen around the common trunk of the bundle. In the late foetus and the adult the entire atrio-ventricular system is invested with a fibrous tissue sheath. When the Purkinje fibre becomes continuous with a myocardial cell the sheath of the fibre becomes continuous with the endomysium of the myocardial cell. The view of Glomset & Glomset (1940b), that the sheath of the conducting tissue separates even the terminal ramifications of the bundle from the myocardium cannot be supported by the observations in this work. Lhamon (1912) and King (1916) were early workers/

workers who directed attention to the sheath and used it to demonstrate the system by the injection technique.

The Purkinje Fibres.

This network is differentiated as extensions of the peripheral branches of the atrio-ventricular bundle. The observation has been made that the first Purkinje cells to appear are sub-endocardial and that the intramyocardial plexus appears slightly later. Abramson and Margolin (1936) called attention to this profuse intramyocardial network, and their findings were amply confirmed during the study of the later foetal and adult hearts. A connexion between the terminal ramifications of the right and left branches of the bundle, through the interventricular septum was reported by Cardwell and Abramson (1931). As reported in the Observations an unsuccessful attempt was made to confirm, histologically, this observation, which was made by means of the injection technique.

The histological continuity of the right branch of the bundle, the sub-endocardial plexus and the intramyocardial network could be demonstrated in a 210 mm. specimen. Field (1951a) was unable to trace these continuities; he attempted to trace them in a 100 mm. specimen and his difficulty/

difficulty may have been exaggerated by the poor differentiation of the Purkinje fibres at 100 mm.

There is general agreement that mitotic division does not occur in the adult Purkinje fibres, and the author is not aware of an illustration of such a division in the literature. The presence of double nuclei suggests the possibility that an amitotic form of division occurs in the Purkinje fibres. Sanabria (1936, P.49, Fig.X) illustrates the different forms which the nucleus of the Purkinje fibre could adopt; these different forms could be the intermediate stages of an amitotic division, but Sanabria rightly asserts that they could also be due to superimposition, amoeboid movement of the nuclear substance or to partial section of a bilobed nucleus. Sanabria (1936, p.48) summarises this subject in these words "Pour ma part, il m'a toujours été impossible, malgré les nombreuses préparations que j'ai pu examiner, de rencontrer la moindre trace de division amitotique". Sanabria did, however, describe the mitotic division of the embryonic Purkinje cell. The author agrees with Field (1951a) who did not see a mitotic division in any cell embryonic or adult that he would regard as of the Purkinje type. Field did decide that amitotic division was probable, but the author would/

would prefer to leave the problem undecided while only fixed preparations are available. Todd and van der Stricht (1920) describe an amitotic division by longitudinal fission in the human Purkinje fibre, and they assert that this mode of division is used for the replacement of the myocardium especially in pathological processes.

Although general conclusions should not be drawn after the examination of one aspect of the system in one species of mammal, it was considered that the facts listed below should be included in any general review of the conducting system.

(i) The conducting system of pre-natal life is limited to a single sinu-atrial node and the atrio-ventricular conducting apparatus.

(ii) The system appears early and develops in a constant and definite manner.

(iii) The presence of this differentiated muscle can not be ascribed to its location in fibrous tissue or to the failure of this muscle to be incorporated in the musculatures of the atria and ventricles. The conducting system is well-differentiated before the advent of fibrous tissue and before the musculatures of the cavities are well-developed.

(iv)/

(iv) The relative size of the conducting system diminishes in the course of development.

(v) The common trunk of the atrio-ventricular bundle is formed before the destruction of the original atrio-ventricular connexions.

(vi) The Purkinje network is formed by a downward spread of the differentiation into the ventricles.

(vii) The conducting system is evident before the nervous tissue, as demonstrated by non-specific methods, has invaded the heart.

(viii) The conducting tissue appears when the heart has acquired a structure which would allow it to function in its adult manner. If the assumption is made that structure is allied to function, then the conducting tissue must take some part in the normal function of the four-chambered heart.

SUMMARY/

SUMMARY

1. The investigation has been carried out on a series of 41 pre-natal and 6 adult sheep hearts.

2. The first portion of the conducting system to appear was the atrio-ventricular bundle, at 6.5 mm. The presence of this differentiated bundle, before the destruction of the original atrio-ventricular connexions, was demonstrated.

3. The atrio-ventricular node was evident at 8.5 mm., but it did not have a definite nodal structure until 20 mm.

4. The main and terminal branches of the bundle differentiated from the ventricular myocardium by a peripheral extension of the differentiation from the common trunk.

5./

5. The sinu-atrial node showed histological differentiation at 10 mm., and in all the later stages it was a constant and definite structure.

6. From the 40 mm. stage onwards, nervous elements were seen in relation to the entire system; the significance of these tissues in relation to the conducting system was discussed.

7. The precocious appearance of the conducting system, and its prominence in the embryo were held to indicate the functional importance of these structures from an early stage in development.

ACKNOWLEDGMENTS

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The preparation of the sections and of the photomicrographs was performed by the author.

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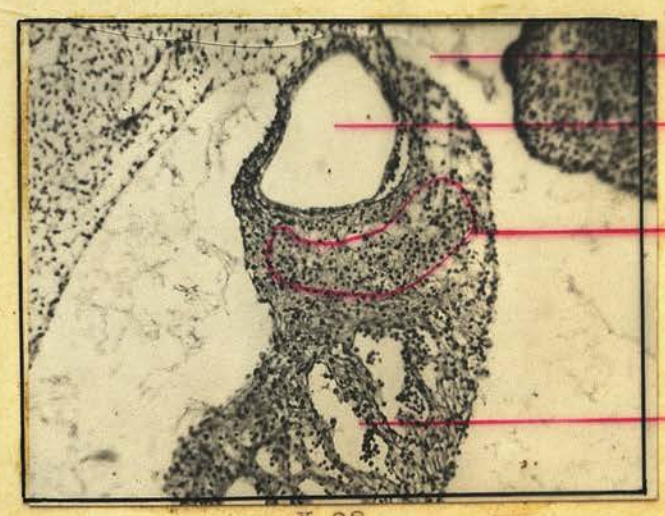
F I G U R E S

All the illustrations are untouched photomicrographs.

Figure 1. - 10.6mm., T.S., Number 5.3.12.

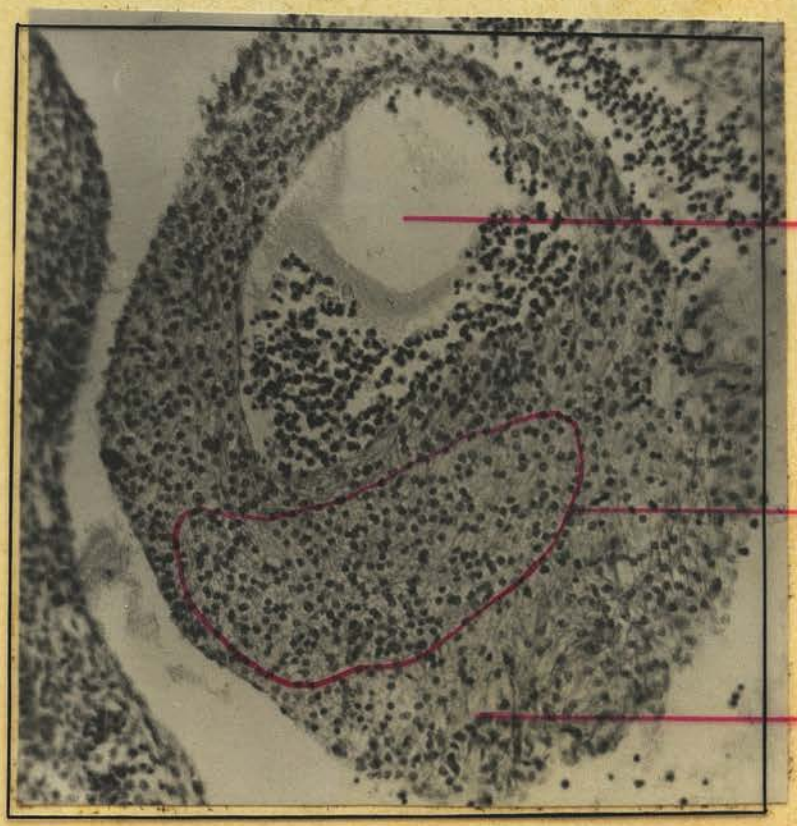
X 12

Figure 2.



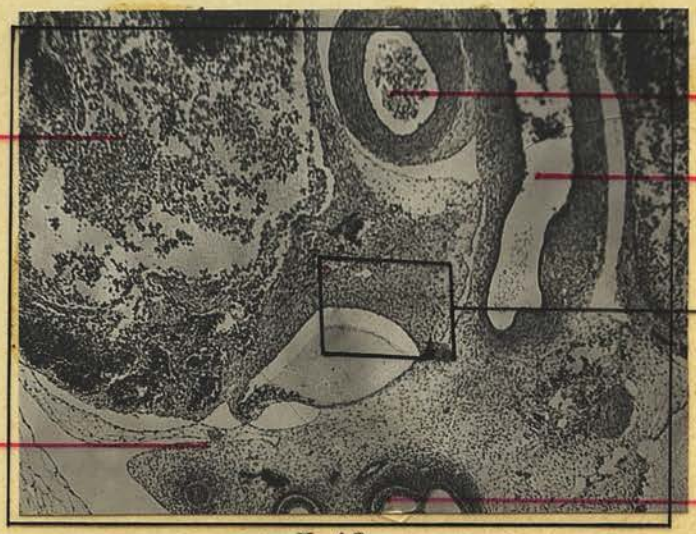
X 92

Figure 3.- 11.7mm., Coronal to embryo, Number 28.2.1.



X 190

Figure 4. - 22.8mm., T.S., Number 14.2.3.

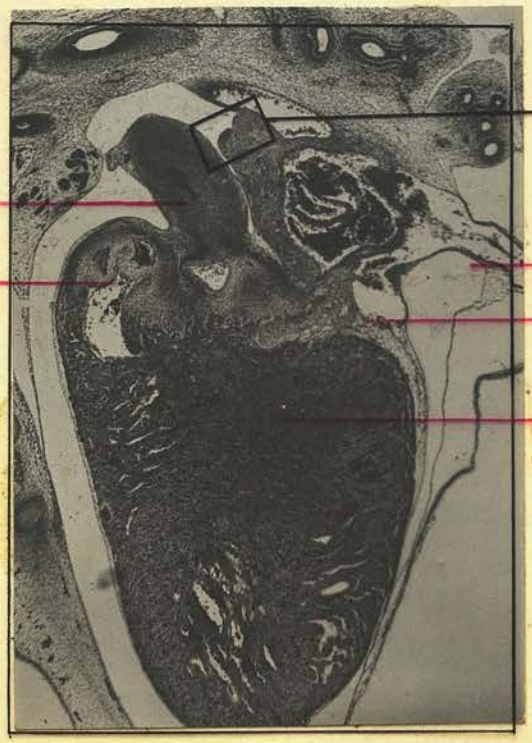


X 42

Figure 5.

X 200

Figure 6. - 25.0mm., Sagittal, Number 13.2.2.

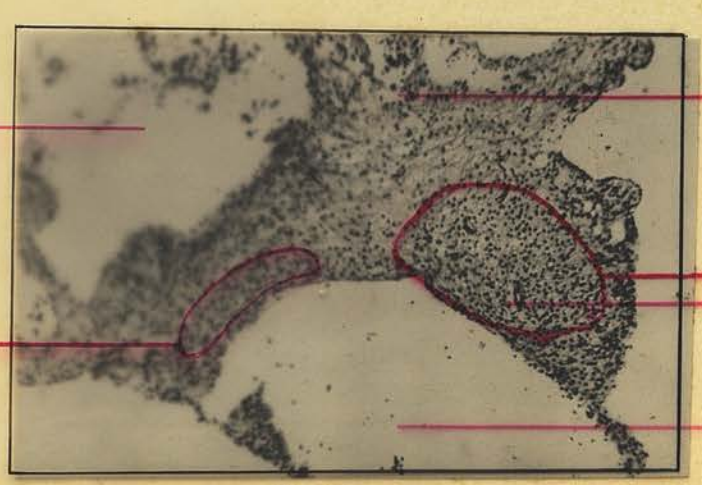


X 20

Figure 7.

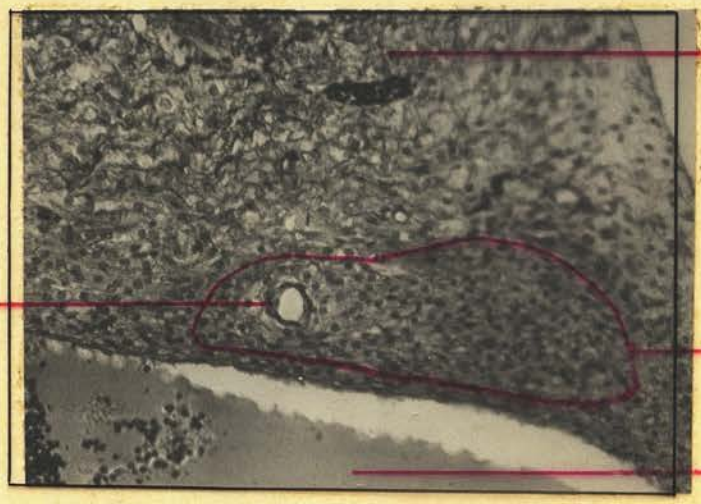
X 200

Figure 8.- 28mm.,T.S. to heart,Number 10.1.11.



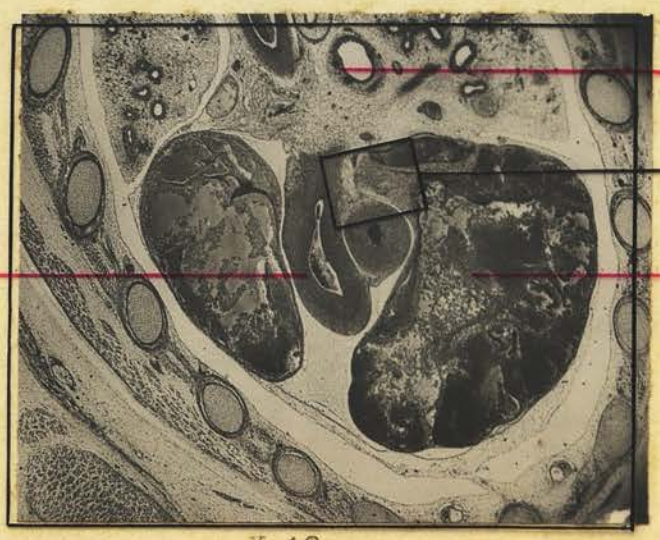
X 92

Figure 9.- 38.6mm.,T.S. to thorax, Number 74.1.2.



X 200

Figure 10.- 41.9mm.,T.S. to thorax,Number 44.1.2.

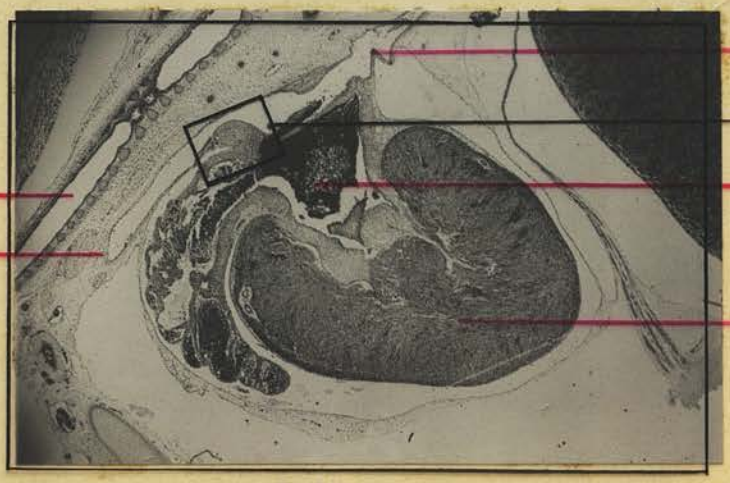


X 12

Figure 11.

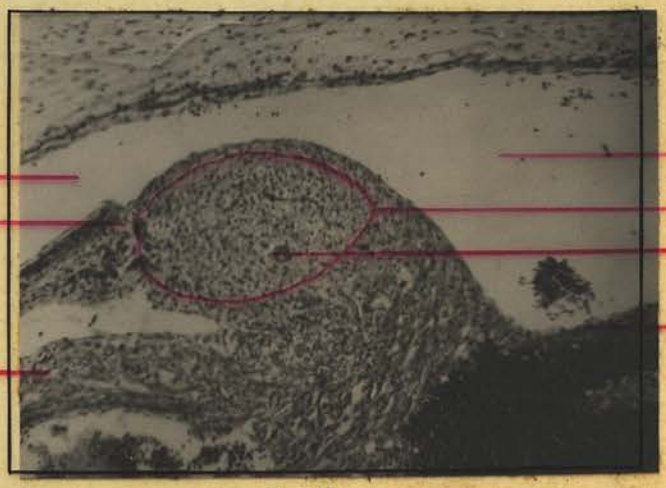
X 92

Figure 12.- 44mm., Sagittal, Number 31.1.5.



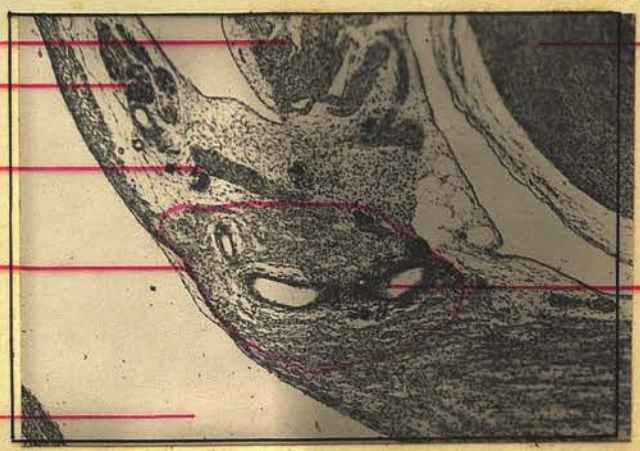
X 10

Figure 13.



X 95

Figure 14.- 136mm., T.S. to heart, Number 56.2.5.



X 40

Figure 15.- 155mm., T.S.to heart, Number 145.1.1.

X 40

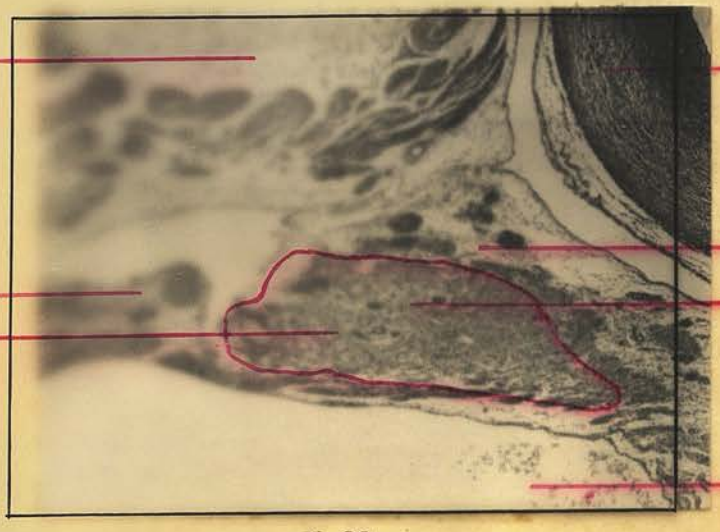
Figure 16.

X 200

Figure 17. - 210mm.,T.S. to heart, Number 1570.

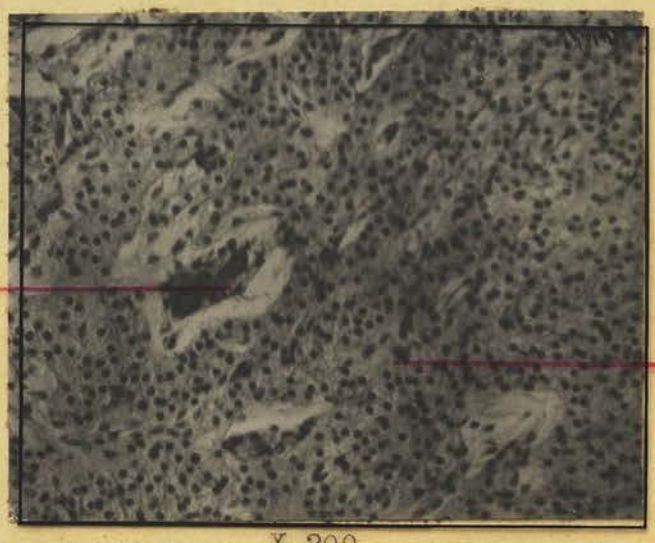
X 3

Figure 18.



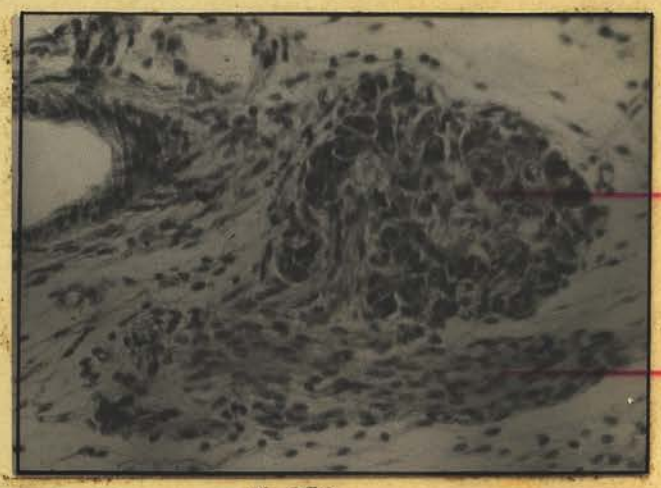
X 28

Figure 19.- 210mm., Portion of the sinu-atrial node.



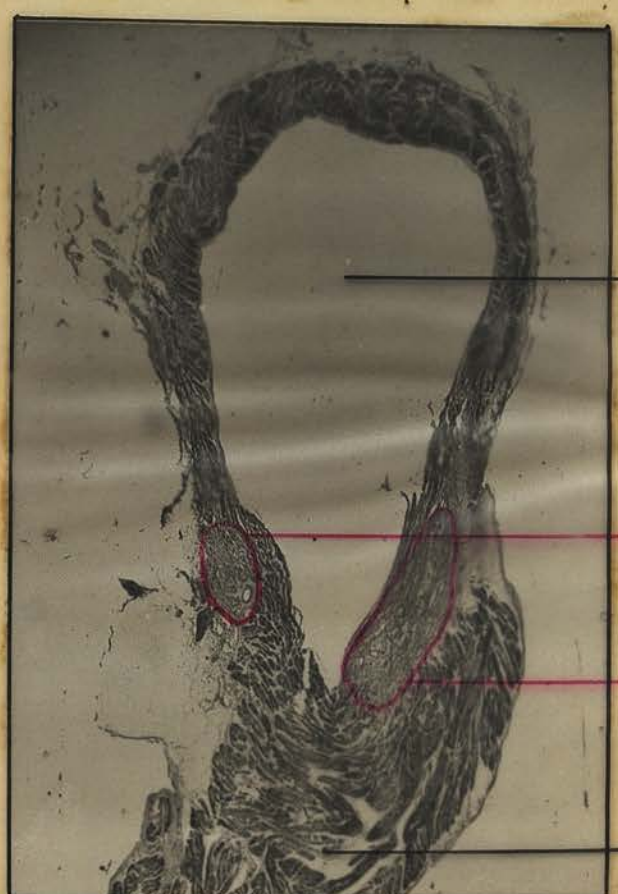
X 200

Figure 20.- 210mm., From the periphery of the sinu-atrial node.



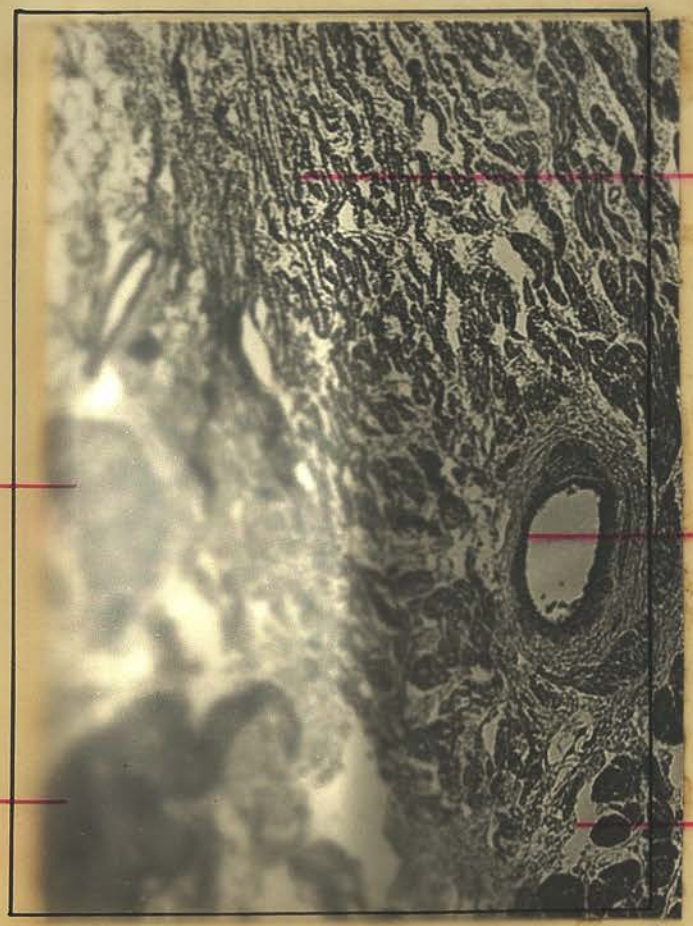
X 230

Adult Sinu-atrial Node.- Number 695



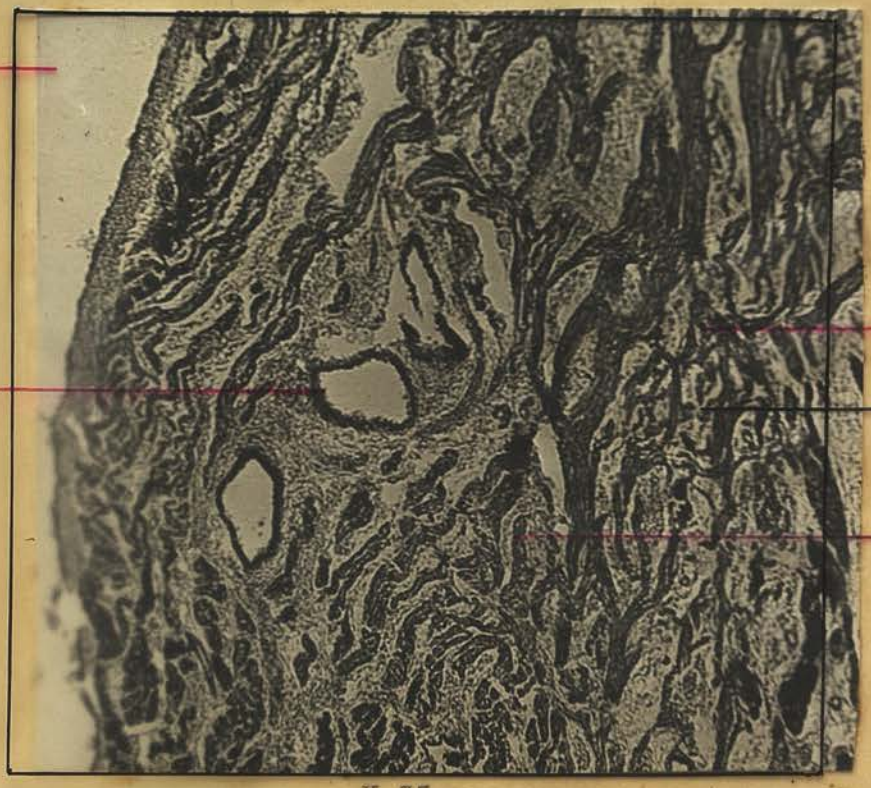
X 3

Adult Sinu-atrial Node.- (a) - Number 695



X 35

Adult Sinu-atrial Node.- (b) - Number 695



X 35

Adult Sinu-atrial Node.- (c) - Number 695.

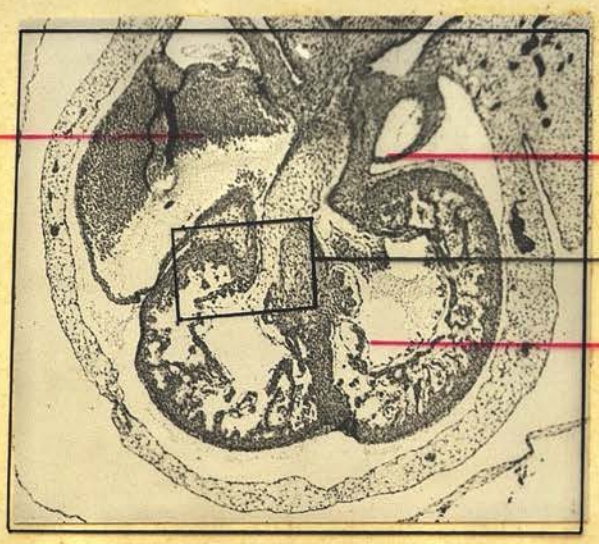
Nodal Fibres

X 600

Normal Atrial Muscle

X 600

Figure 21. - 6.7mm.,T.S.,Number 5.2.8.



X 40

Figure 22.

X 200

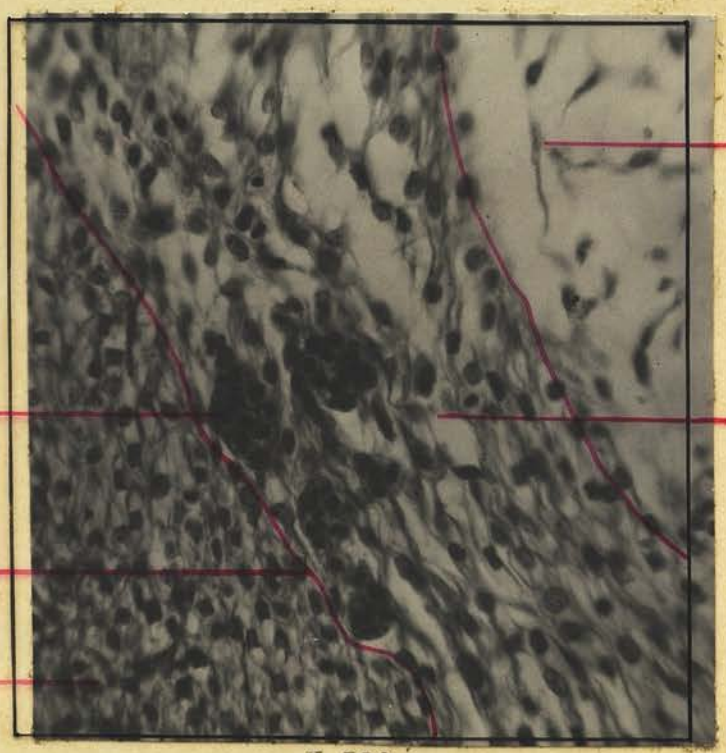
Figure 23. - 8.4mm., Sagittal, Number 10.1.3.

X 34

Figure 24.

X 92

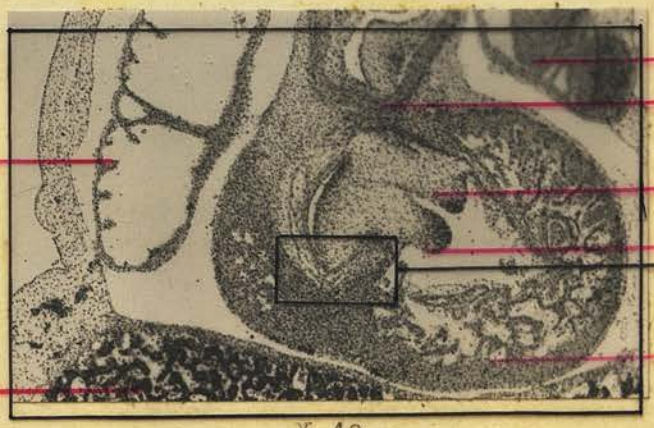
Figure 25.- 8.4mm., Sagittal, Number 10.2.2.



X 380

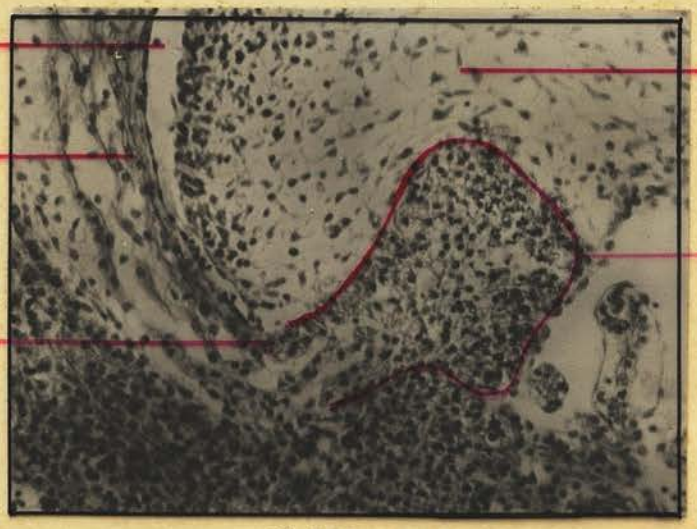
Compare with Figure 23.

Figure 26.- 9.1mm., Coronal to embryo, Number 11.2.5.



X 40

Figure 27.

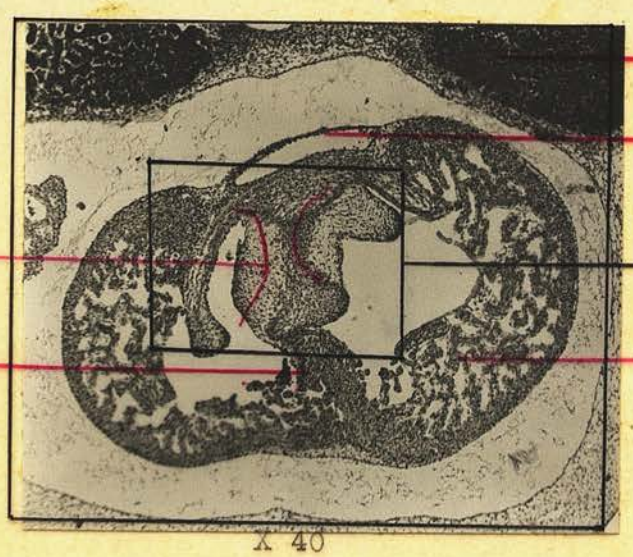


X 200

Figure 28. - 10.6mm.,T.S.,Number 7.2.11.

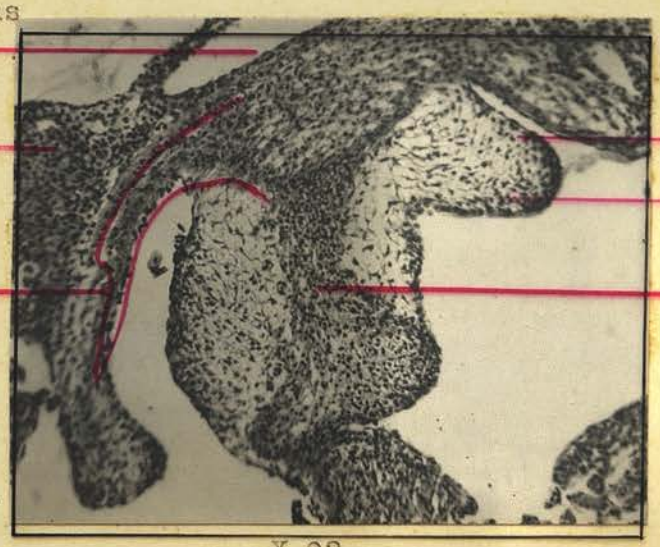
X 10

Figure 29.



X 40

Figure 30.- See Figures 28 & 29.



X 92

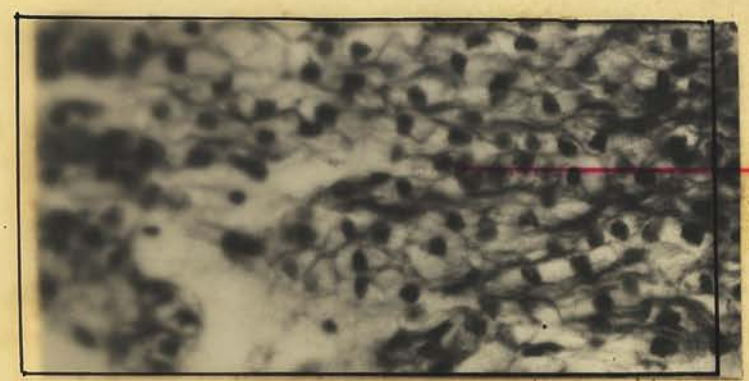
Figure 31.- 11.6mm.,Coronal, Number 9.2.11.

X 92

Figure 32.- 18.4mm., Sagittal, Number 16.2.4.

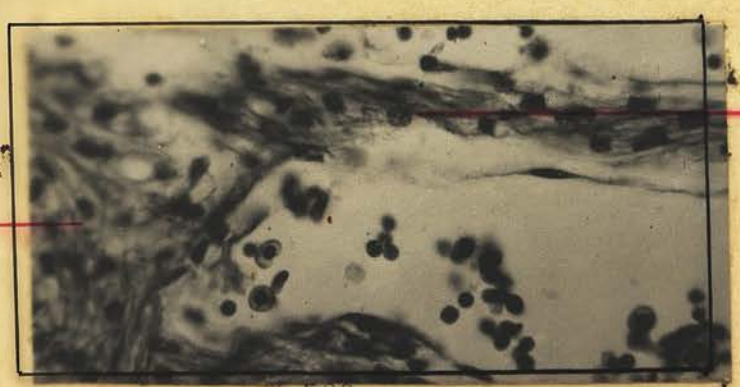
X 92

Figure 33.



X 500

Figure 34.

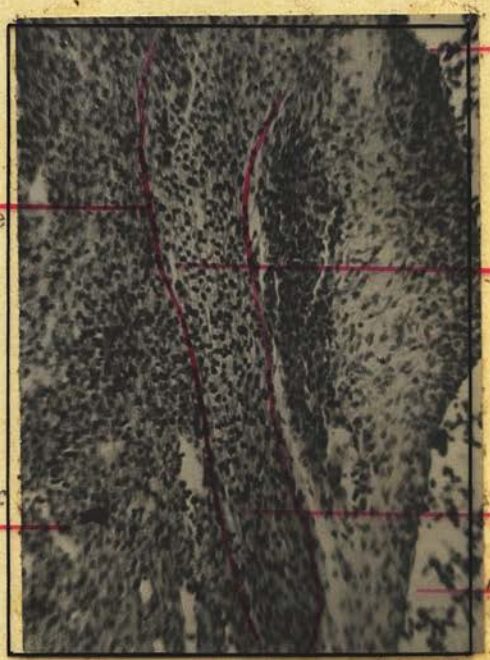


X 500

Figure 35.- 20.1 mm., T.S. to thorax, Number 5.1.8.

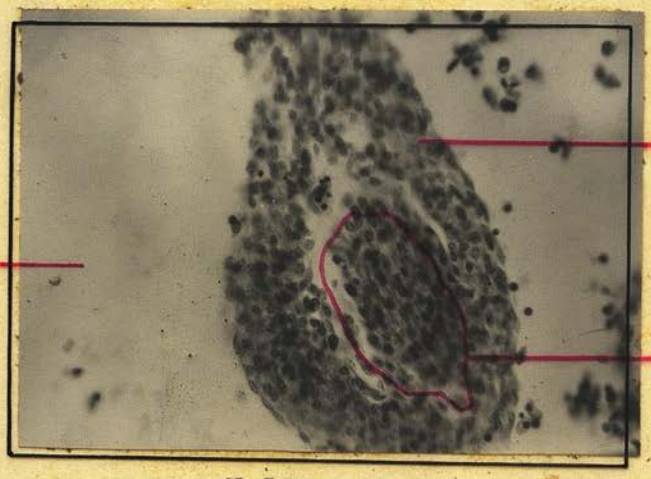
X 10

Figure 36.-



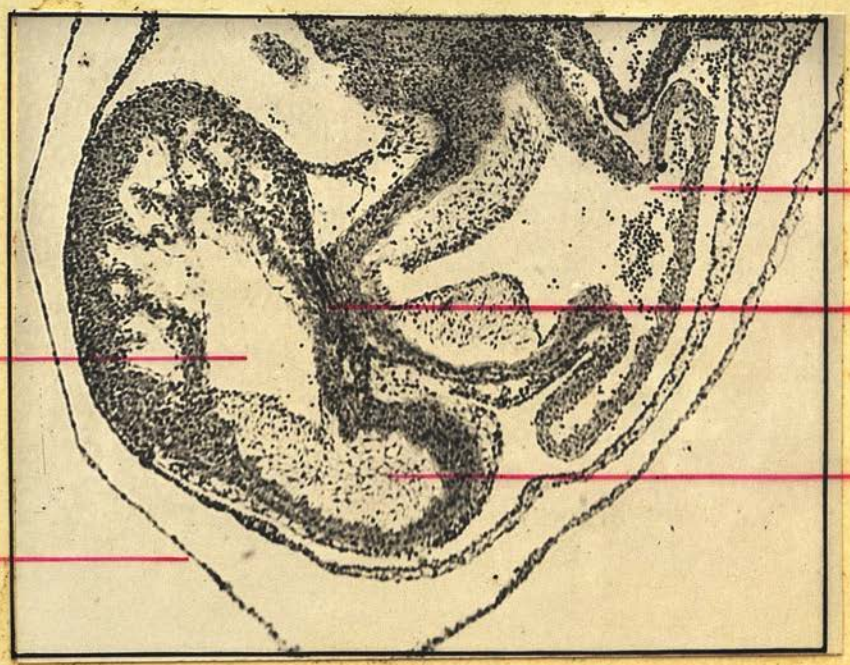
X 120

Figure 37.- 20.1mm., T.S. to thorax, Number 4.2.7.



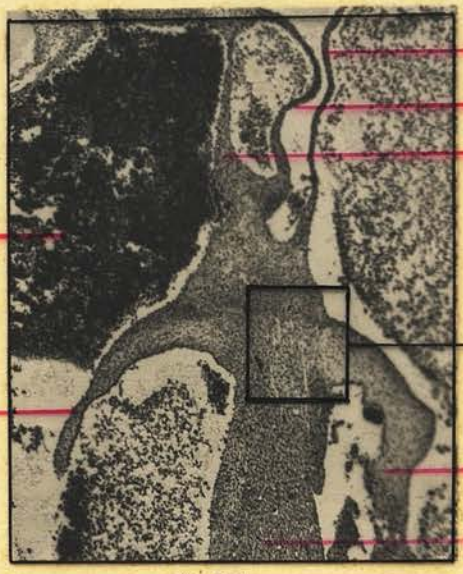
X 320

Figure 38.- 5.4mm., T.S. to embryo, Number 2.4.5.



X 28

Figure 39.- 22.8mm., T.S. to thorax, Number 8.5.8.

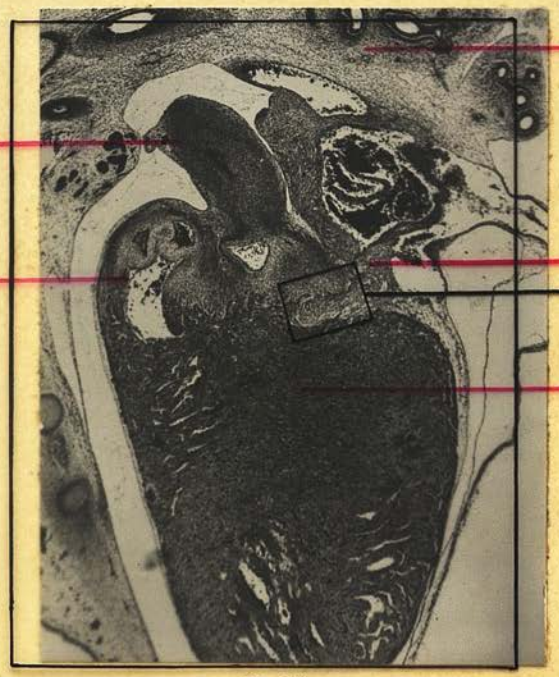


X 42

Figure 40.

X 200

Figure 41.- 25mm., Sagittal, Number 13.2.2.

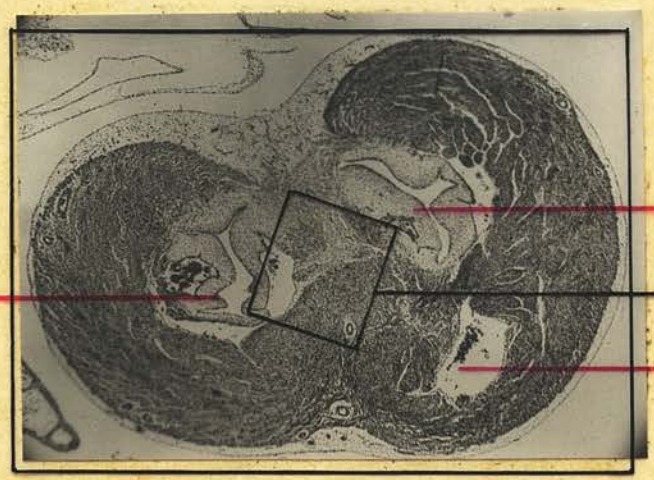


X 20

Figure 42.

X 200

Figure 43.- 28mm.,T.S. to heart,Number 6.2.5.



X 22

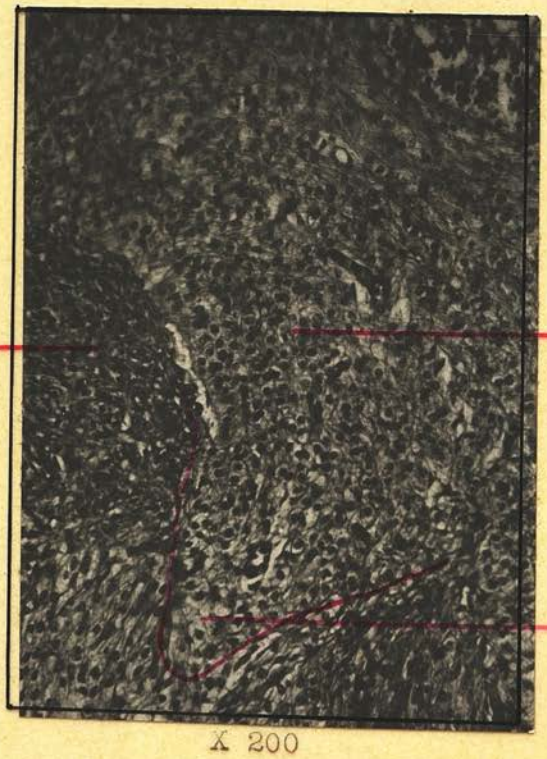
Figure 44.

X 92

Figure 45.- 38.6mm.,T.S. to heart, Number 48.1.6.

X 40

Figure 46.

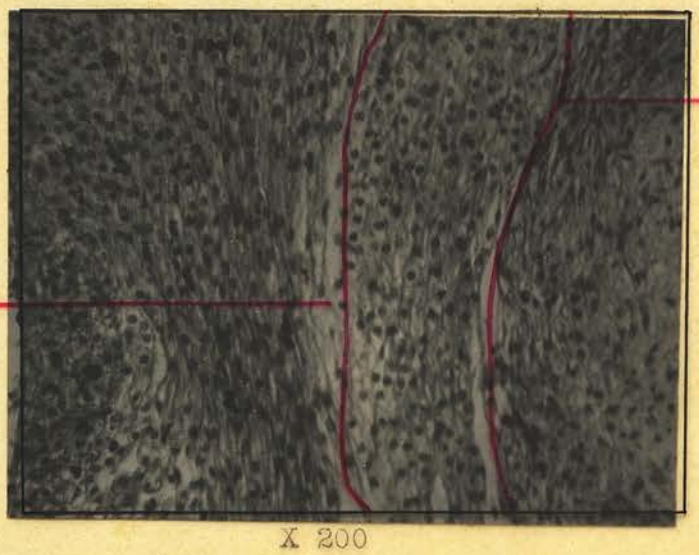


X 200

Figure 47.- 41.9mm.,T.S.to thorax, Number 28.1.2.

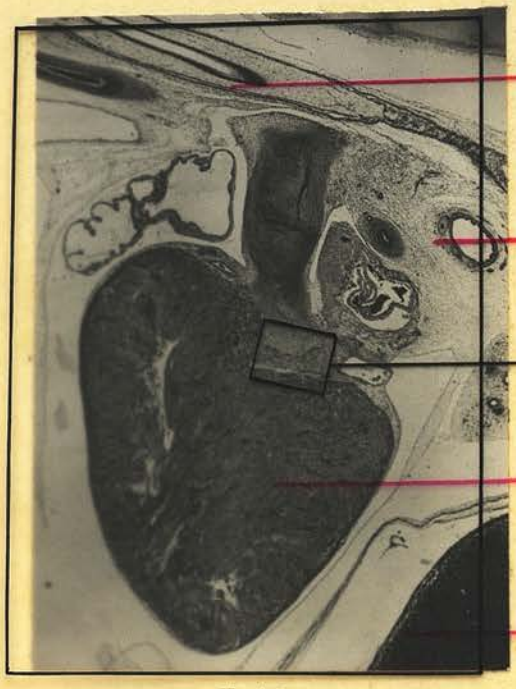
X 12

Figure 48.



X 200

Figure 49.- 44mm., Sagittal, Number 25.2.3.

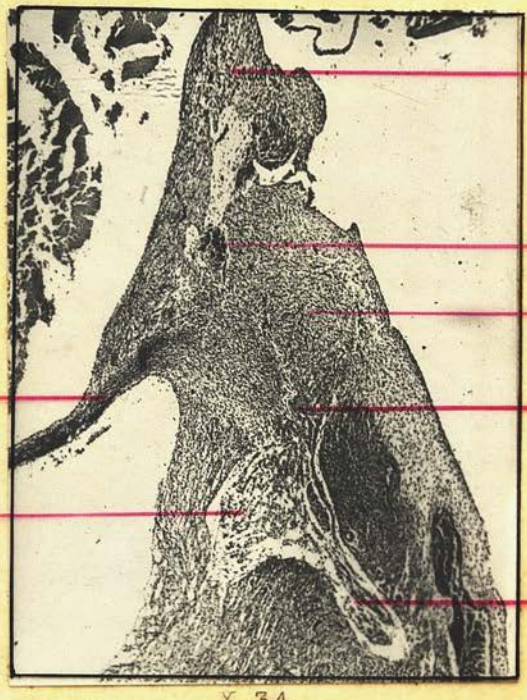


X 10

Figure 50.

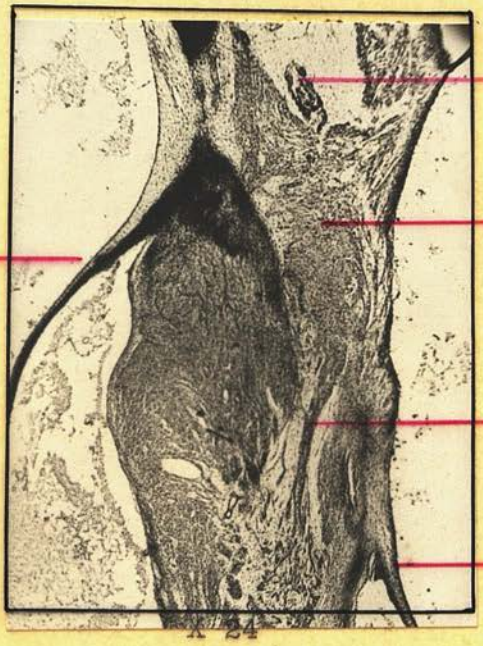
X 92

Figure 51.- 92mm., T.S. to heart, Number 8.1.6.



X 34

Figure 52.- 138mm., T.S. to heart, Number 39.1.3.



X 24

Figure 53.- 155mm.,T.S. to heart, Number 80.1.3.

X 24

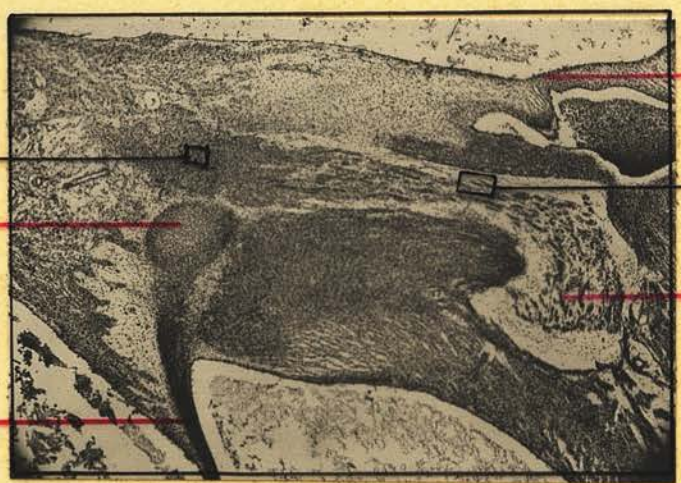
Figure 54.

X 92

Figure 55.- 210mm., T.S. to heart, Number 880

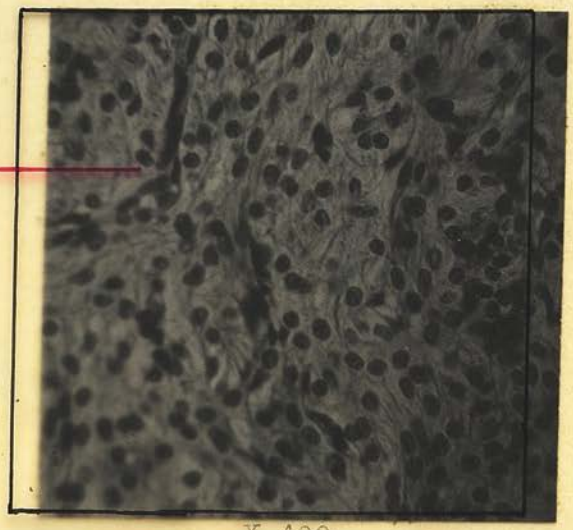
X 3

Figure 56.



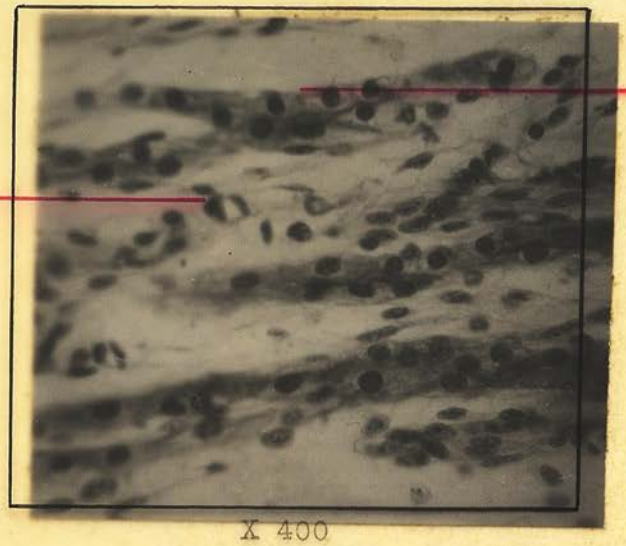
X 24

Figure 57.- Atrio-ventricular Node, 210mm.



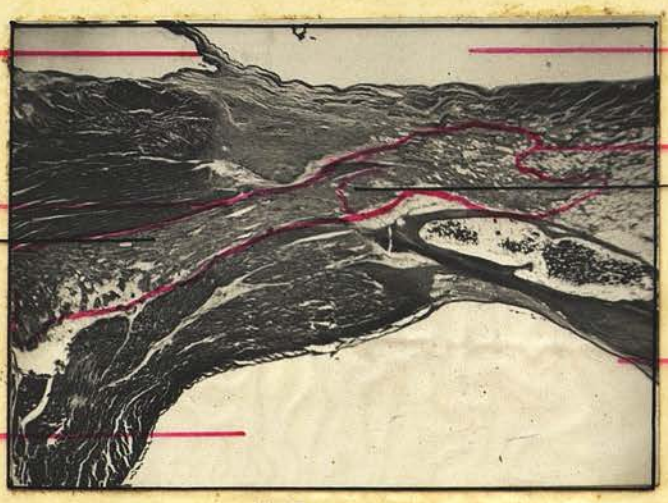
X 400

Figure 58.- Atrio-ventricular Bundle, 210mm.



X 400

Adult Atrio-Ventricular Node and Bundle



X 3

(a)

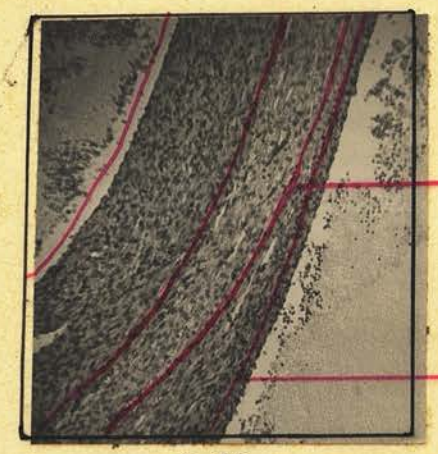
X 380

(b)

Figure 59.- 44mm., Sagittal, Number 27.1.3.

X 92

Figure 60.- 63mm., T.S. to heart, Number 6.1.7.



X 92

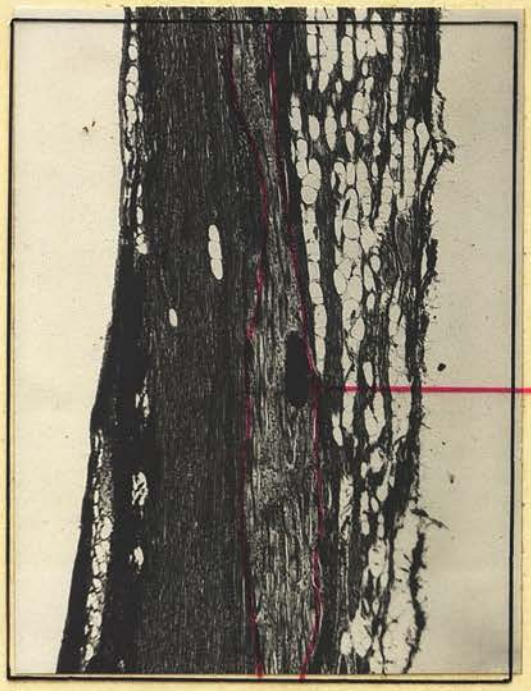
Figure 61.- Moderator Band, 335mm.

X 40

Figure 62.

X 400

Figure 63.- Moderator Band, Adult



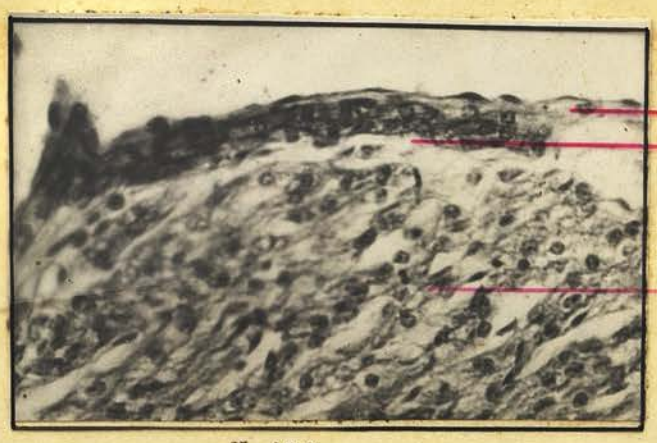
X 37

Figure 64.

X 400

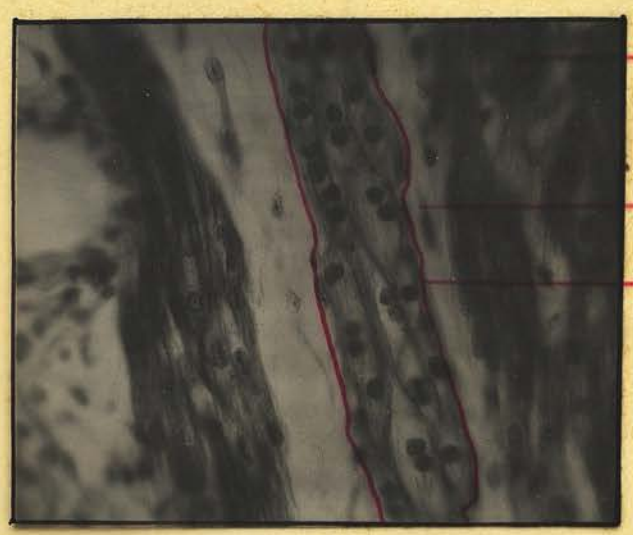
X 400

Figure 65.- 71mm.,T.S. to heart, Number 4.4.6.



X 480

Figure 66.- 210mm., T.S. to heart, Number 880.



X 400